



CONSIDERATION OF FUTURE DISCUSSION OF UNUSUAL CASES OF SPONGIFORM ENCEPHALOPATHY IN CATTLE

ISSUE

1. To consider the scope of a future discussion of data on unusual cases of spongiform encephalopathy in cattle.

BACKGROUND

2. In the past few years, a number of cases of spongiform encephalopathy have occurred in predominantly older cattle with neuropathological phenotypes distinct from bovine spongiform encephalopathy (BSE). These cases have been identified from active surveillance programmes in a number of European countries, in Japan and the United States of America (USA). To date, no such cases have been identified from surveillance in the United Kingdom. Investigations of the cases show differences in the pattern of deposition and regional distribution of abnormal prion protein (PrP^{Sc}) in the brain and in banding patterns on western blot analysis compared with those expected for BSE. Transmission studies have shown that the spongiform encephalopathy is transmissible to mice, with characteristics distinguishable from BSE. The findings suggest that cattle may be susceptible to another, or other strains of naturally occurring transmissible spongiform encephalopathy (TSE).
3. Some researchers have described these unusual cases as 'atypical BSE'. However, SEAC previously expressed the opinion that use of the term 'atypical BSE' was confusing and should be discouraged. One research group has distinguished the cases it has investigated from BSE by describing the condition as bovine amyloidotic spongiform encephalopathy (BASE). It is suggested that this definition is used in subsequent discussions.
4. The intergovernmental TSE Research and Development Joint Funders Coordination Group has suggested that it would be timely for SEAC to consider the scientific information on unusual BSE cases. In preparation for such a discussion the SEAC secretariat

has conducted a search¹ to identify relevant published literature and researchers conducting investigations of this phenomenon (see Annex 1 and 2). Relevant Defra funded research projects are given at Annex 3.

PREVIOUS SEAC CONSIDERATION

5. At SEAC 81, SEAC considered the findings of a research paper² suggesting that a second bovine spongiform encephalopathy exists with a molecular signature similar to that of a subtype of sporadic CJD. SEAC agreed that the results were very interesting, but without information on the transmissibility, it was premature at this stage to conclude this was a new strain of BSE. SEAC agreed it would follow this research with interest.
6. At SEAC 93, SEAC noted reports that western blot profiles of two cases of BSE in USA cattle were similar to a small number of unusual cases of BSE found in France. A study³ of the French cases had shown the condition to be transmissible to mice by intracerebral inoculation, with the neuropathological phenotype maintained on transmission. Claims had been made about the existence of further such cases in cattle that are distinct from BSE in other countries. No study had examined the tissue distribution of PrP^{Sc} or infectivity in such cases.

ADVICE SOUGHT FROM THE COMMITTEE

7. The committee is invited to:
 - agree to a discussion of BASE at SEAC 97.
 - identify any other relevant published research not included in this paper (Annexes 1 and 3) to form the basis of a review.
 - identify experts that should be invited to present unpublished research to the committee and take part in a discussion of BASE (Annex 2).

¹ Articles are searched through Entrez, The Life Science Search Engine, using the search terms such as atypical BSE, atypical TSE and bovine amyloidotic spongiform encephalopathy.

² Casalone C *et al.* Identification of a second bovine amyloidotic spongiform encephalopathy: molecular similarities with sporadic Creutzfeldt-Jakob disease. *Proc Natl Acad Sci U S A.* 2004 Mar 2;101(9):3065-70.

³ Baron *et al.* (2006) Transmission of new bovine prion to mice. *Emerging. Infect. Diseases.* 12, 1125-1128.



ABSTRACTS RELATED TO BASE

Abstracts of published papers (in chronological order)

1. **Baron TG, Biacabe AG, Bencsik A, Langeveld JP. Transmission of new bovine prion to mice. *Emerg Infect Dis.* 2006 Jul;12(7):1125-8.**

We previously reported that cattle were affected by a prion disorder that differed from bovine spongiform encephalopathy (BSE) by showing distinct molecular features of disease-associated protease-resistant prion protein (PrP(res)). We show that intracerebral injection of such isolates into C57BL/6 mice produces a disease with preservation of PrP(res) molecular features distinct from BSE.

2. **Baron T, Biacabe AG, Arzac JN, Benestad S, Groschup MH. Atypical transmissible spongiform encephalopathies (TSEs) in ruminants. *Vaccine.* 2006 Nov 13; [Ahead of print]**

Transmissible spongiform encephalopathies (TSEs) are associated with the accumulation in infected tissues of a disease-associated form of a host-encoded protein, the prion protein (PrP). Contrary to the normal form of the protein, this form of PrP is partially resistant to protease digestion (PrP(res)). Detailed characterisation of PrP(res) has been intensively investigated in recent years to try and decipher the diversity of TSEs in human and animals. This considerably and unexpectedly enlarged our knowledge about such diseases in ruminants. Previously, such a diversity was essentially shown by the demonstration that scrapie from sheep and goats could have different biological behaviours following transmission of the disease in mice, unlike bovine spongiform encephalopathy from cattle (BSE) which showed a distinct and unique behaviour. The properties of the BSE agent were also demonstrated to be very stable, following transmission to a variety of different species. Molecular studies of PrP(res), followed by transmission studies to

mice, gave the first evidence for the accidental transmission of the BSE agent to humans where it induced a variant form of the fatal Creutzfeldt-Jakob disease (CJD) and also to different animal species including a goat in France. This last case was found among a few unusual cases of TSEs in small ruminants that showed some molecular similarities with BSE and which are currently under investigation by transmission studies in mice. The application of the molecular methods to characterise PrP(res) has most recently led to the unexpected discovery of deviant BSE forms in a few affected cattle in Europe and in the United States, which raises the question of a possible different origin at least of some cases of BSE in cattle. Finally, considerable numbers of a new TSE form in small ruminants, referred to as "atypical scrapie" or "Nor98", have meanwhile been identified in most European countries by TSE rapid testing using an assay which recognizes also comparatively less PK resistant PrP(res).

3. ***Beringue V, Bencsik A, Le Dur A, Reine F, Lai TL, Chenais N, Tilly G, Biacabe AG, Baron T, Vilotte JL, Laude H. Isolation from cattle of a prion strain distinct from that causing bovine spongiform encephalopathy. PLoS Pathog. 2006 Oct;2(10):e112.***

To date, bovine spongiform encephalopathy (BSE) and its human counterpart, variant Creutzfeldt-Jakob disease, have been associated with a single prion strain. This strain is characterised by a unique and remarkably stable biochemical profile of abnormal protease-resistant prion protein (PrP(res)) isolated from brains of affected animals or humans. However, alternate PrP(res) signatures in cattle have recently been discovered through large-scale screening. To test whether these also represent separate prion strains, we inoculated French cattle isolates characterised by a PrP(res) of higher apparent molecular mass--called H-type--into transgenic mice expressing bovine or ovine PrP. All mice developed neurological symptoms and succumbed to these isolates, showing that these represent a novel strain of infectious prions. Importantly, this agent exhibited strain-specific features clearly distinct from that of BSE agent inoculated to the same mice, which were retained on further passage. Moreover, it also differed from all sheep scrapie isolates passaged so far in ovine PrP-expressing mice. Our findings therefore raise the possibility that either various prion strains may exist in cattle, or that the BSE agent has undergone divergent evolution in some animals.

4. ***Biacabe AG, Laplanche JL, Ryder S, Baron T. Distinct molecular phenotypes in bovine prion diseases. EMBO Rep. 2004 Jan;5(1):110-5.***

Intensive active surveillance has uncovered two atypical German BSE cases in older cattle which resemble the two different atypical BSE phenotypes that have recently been described in France (designated H-type) and Italy (designated L-type or BASE). The H-type is characterized by a significantly higher molecular size, but a conventional glycopattern of the proteinase K treated abnormal prion protein (PrP(Sc)), while the L-type PrP(Sc) has only a slightly lower molecular size and a distinctly different glycopattern. In this paper we describe the successful transmission of both German atypical BSE cases to transgenic mice overexpressing bovine PrP(C). Upon challenge with the L-type, these mice developed BSE after a substantially shorter incubation period than any classical BSE transmission using these mice to date. In contrast, the incubation period was distinctly prolonged when these mice were challenged with the H-type. PrP(Sc) accumulated in the brains of these mice were of the same atypical BSE type that had been used for the transmission. These atypical cases suggest the possible existence of sporadic BSE cases in bovines. It is thus feasible that the BSE epidemic in the UK could have also been initiated by an intraspecies transmission from a sporadic BSE case.

5. ***Buschmann A, Gretzschel A, Biacabe AG, Schiebel K, Corona C, Hoffmann C, Eiden M, Baron T, Casalone C, Groschup MH. Atypical BSE in Germany--proof of transmissibility and biochemical characterization. Vet Microbiol. 2006 Oct 31;117(2-4):103-16.***

Intensive active surveillance has uncovered two atypical German BSE cases in older cattle which resemble the two different atypical BSE phenotypes that have recently been described in France (designated H-type) and Italy (designated L-type or BASE). The H-type is characterized by a significantly higher molecular size, but a conventional glycopattern of the proteinase K treated abnormal prion protein (PrP(Sc)), while the L-type PrP(Sc) has only a slightly lower molecular size and a distinctly different glycopattern. In this paper we describe the successful transmission of both German atypical BSE cases to transgenic mice overexpressing bovine PrP(C). Upon challenge with the L-type, these mice developed BSE after a substantially shorter incubation period than any classical BSE transmission using these mice to date. In contrast, the incubation period was distinctly prolonged when these mice were challenged with the H-type. PrP(Sc) accumulated in the

brains of these mice were of the same atypical BSE type that had been used for the transmission. These atypical cases suggest the possible existence of sporadic BSE cases in bovines. It is thus feasible that the BSE epidemic in the UK could have also been initiated by an intraspecies transmission from a sporadic BSE case.

6. **Casalone C, Zanusso G, Acutis P, Ferrari S, Capucci L, Tagliavini F, Monaco S, Caramelli M. Identification of a second bovine amyloidotic spongiform encephalopathy: molecular similarities with sporadic Creutzfeldt-Jakob disease. Proc Natl Acad Sci U S A. 2004 Mar 2;101(9):3065-70.**

Transmissible spongiform encephalopathies (TSEs), or prion diseases, are mammalian neurodegenerative disorders characterized by a posttranslational conversion and brain accumulation of an insoluble, protease-resistant isoform (PrP(Sc)) of the host-encoded cellular prion protein (PrP(C)). Human and animal TSE agents exist as different phenotypes that can be biochemically differentiated on the basis of the molecular mass of the protease-resistant PrP(Sc) fragments and the degree of glycosylation. Epidemiological, molecular, and transmission studies strongly suggest that the single strain of agent responsible for bovine spongiform encephalopathy (BSE) has infected humans, causing variant Creutzfeldt-Jakob disease. The unprecedented biological properties of the BSE agent, which circumvents the so-called "species barrier" between cattle and humans and adapts to different mammalian species, has raised considerable concern for human health. To date, it is unknown whether more than one strain might be responsible for cattle TSE or whether the BSE agent undergoes phenotypic variation after natural transmission. Here we provide evidence of a second cattle TSE. The disorder was pathologically characterized by the presence of PrP-immunopositive amyloid plaques, as opposed to the lack of amyloid deposition in typical BSE cases, and by a different pattern of regional distribution and topology of brain PrP(Sc) accumulation. In addition, Western blot analysis showed a PrP(Sc) type with predominance of the low molecular mass glycoform and a protease-resistant fragment of lower molecular mass than BSE-PrP(Sc). Strikingly, the molecular signature of this previously undescribed bovine PrP(Sc) was similar to that encountered in a distinct subtype of sporadic Creutzfeldt-Jakob disease.

7. **Yamakawa Y, Hagiwara K, Nohtomi K, Nakamura Y, Nishijima M, Higuchi Y, Sato Y, Sata T. Expert Committee for BSE Diagnosis, Ministry of Health, Labour and Welfare of Japan.**

Atypical proteinase K-resistant prion protein (PrPres) observed in an apparently healthy 23-month-old Holstein steer. Jpn J Infect Dis. 2003 Oct-Dec;56(5-6):221-2

An ELISA-positive specimen from a 23 month-old Holstein steer slaughtered on September 29, 2003, in Ibaraki Prefecture (Ibaraki case) was sent to the NIID for confirmation. The animal was reportedly healthy before slaughter. The OD titer in ELISA was slightly higher than the 'cut-off' level given by the manufacturer. The histology showed no spongiform changes and IHC revealed no signal of PrP^{Sc} accumulation typical for BSE. However, WB analysis of the homogenate that was prepared from the obex region and used for ELISA revealed a small amount of PrP^{Sc} with an electrophoretic profile different from that of typical BSE-associated PrP^{Sc}. The characteristics were (i) low content of the diglycosylated molecular form of PrP^{Sc}, (ii) a faster migration of the non-glycosylated form of PrP^{Sc} on SDS-PAGE, and (iii) less resistance against PK digestion as compared with an authentic PrP^{Sc} specimen derived from an 83-month-old Holstein (Wakayama case).

Abstracts from the meeting presentations.

8. ***Tagliavini F, Capobianco R, Casalone C, Miccolo C, Suardi S, Mangieri M, Limido L, Bruzzone MG, Corona C, Zanusso G, Monaco S and Caramelli M. Experimental transmission of an atypical form of bovine spongiform encephalopathy. J Neuropathol Exp Neurol. 2005; V64(N5):p 472.***

A novel BSE phenotype has been recently identified in Italy. This phenotype (termed BASE) differs from typical BSE for the presence of PrP-amyloid plaques and the occurrence of a distinct PrPres type, suggesting that BSE and BASE may be related to different prion strains. To investigate this issue we set up transmission experiments to a panel of four inbred mouse strains, including SJL, C57Bl/6, RIII and VM mice (n=20 animals/group). At the time of writing, complete results are available for SJL and C57Bl/6 mice. The SJL after 280±26 and 267±17 days, respectively, while SJL mice challenged with BASE were culled at 560 days post-inoculation without neurological symptoms. The incubation time of the C57Bl/6 injected with BSE challenged with BASE are still free of neurological symptoms. This clinical difference between groups was paralleled by a difference in the appearance of signal abnormalities at MRI. Neuropathological examination of BSE and vCJD infected mice sacrificed at the terminal stage of disease showed a spongiform encephalopathy with diffuse and plaque like

PrPres deposits, and a PrPres profile with a glycosylated dominant pattern similar to that of BSE. By contrast, no PrPres was detected in mice challenged with BASE. These data support the view that BSE and BASE are caused by strains with different biological properties. The “species barrier” to transmission of BASE seems to be substantially higher than that of BSE.

9. ***Richt JA and Hall SM. Atypical Bovine Spongiform Encephalopathy case associated with a prion protein gene mutation. Book of abstracts, Prion 2006, International conference on Prion Diseases of NeuroPrion, Network of Excellence, Turin, Italy, 3-6 October 200.***

Transmissible spongiform encephalopathy (TSE) agents induce fatal neurodegenerative diseases in humans and in some other mammalian species. Human TSEs include Creutzfeldt–Jakob disease (CJD), Gerstmann-Sträussler-Scheinker (GSS) syndrome, Kuru and Fatal Familial Insomnia (FFI). In animals, several distinct TSE diseases are recognized: scrapie in sheep and goats, transmissible mink encephalopathy (TME) in mink, chronic wasting disease (CWD) in cervids, and bovine spongiform encephalopathy (BSE) in cattle. BSE was first detected in 1986 in the United Kingdom and is the most likely cause of variant CJD in human. The origin of BSE remains an enigma. Hypotheses include (i) scrapie-infected tissues included in cattle rations, (ii) previously undetected sporadic or genetic bovine TSE, or (iii) origination from a human TSE through feed containing mammalian raw materials contaminated with human remains. Here we report a bovine BSE case associated with a novel prion protein gene (*Prnp*) polymorphism resulting in an amino acid substitution. The animal carrying this mutation was positive for the abnormal prion protein, PrPBSE, in brainstem tissue. Western blot characterization of the PrPBSE of this animal revealed a high molecular weight phenotype of BSE when compared to PrPBSE from typical BSE isolates. An identical mutation in the human prion protein sequence homologue has been previously described as the most common cause of genetic human prion diseases (gCJD, FFI and GSS). Studies are underway to develop a single nucleotide polymorphism assay in order to identify this allele in cattle.

10. ***Comoy E, Casalone C, Lescoutra N, Zanusso G, Ferrari S, Marce D, Avure F, Ruchoux MM, Monaco S, Sales N, Brown P, Caramelli M, Deslys JP and Lasmézas C. Transmission of bovine amyloidotic spongiform encephalopathy (BASE) to the primate. Book of abstracts, Prion 2006, International***

conference on Prion Diseases of NeuroPrion, Network of Excellence, Turin, Italy, 3-6 October 2006.

The recent discovery of a novel prion disease apparently sporadic in cattle and classified as Bovine Amyloidotic Spongiform Encephalopathy (BASE), raised the question of its origin and of the risk for man. Based on western blot analyses of the abnormal prion protein (PrPres), molecular similarities have been described between two cases of BASE found in Italy and a subtype of sporadic Creutzfeldt-Jakob disease (CJD) in man. However the assessment of a link between the two diseases was difficult as the biochemical signature of the PrPres originated from very different mammalian species. We report here the result of the transmission of BASE from cattle to primate, using the macaque primate model that is susceptible to both bovine spongiform encephalopathy (BSE) and variant CJD (vCJD). In the animal inoculated intracerebrally with a 10% brain homogenate we observed a transmission of the disease that was lethal within 26 months. The clinical signs and histopathological lesions were different from those observed in BSE or vCJD. This transmission was approximately one third faster than that observed with BSE, despite a substantially lower PrPres amount in the inoculum. The adaptation of a diagnostic test developed for discrimination of prion strains in sheep allowed us to observe a distinct biochemical signature of PrPres with a high proteinase K sensitivity of the octarepeat region. Our observations underline the high susceptibility of a primate species to the BASE prion strain and provide a biochemical basis for the identification of a potential occurrence in man.

11. ***Casalone C, Corona C, Iulini B, Porcario C, Caramelli M, Langeveld JPM, and Van Keulen. Atypical BSE isolates in cattle: Immunohistochemical comparison between Dutch and Italian cases. Book of abstracts, Prion 2006, International conference on Prion Diseases of NeuroPrion, Network of Excellence, Turin, Italy, 3-6 October 2006.***

Unusual isolates of BSE have been reported in the past two years in different European countries and Japan. Two variant forms of BSE have been reported based on biochemical analysis of the molecular mass and glycoform ratio of the pathological prion protein (PrPsc): i) a type with a lower molecular mass of the unglycosylated isoform and a distinct glycopattern of PrPres (L-type or BASE) and (ii) a type with higher molecular mass of the unglycosylated isoform and a distinct glycopattern of PrPres (H-type). From a pathological point of view, a careful description was

only available for the two Italian cases (L-type) where immunohistochemical analysis revealed an unusual PrP^{Sc} deposition pattern characterized by the presence of amyloid plaques particularly in the white matter of olfactory bulb, frontal and parietal cortex. Here we report the results of a comparative immunohistochemical study between an H-type BSE case identified in the Netherlands and the two Italian L-type or BASE cases, using a panel of four different antibodies (9A2, 94B4, 12B2, F99/97.6.1). The antibodies used recognize different linear and conformational epitopes that are dispatched along the PrP sequence. The study conducted at the level of the obex and pons indicates that F99/97.6.1 better discriminates the two different phenotypes.

12. ***Buschmann A, Gretzschel A, Biacabe AG, Schiebel K, Corona C, Hoffmann C, Eiden M, Baron T, Casalone C, Tagliavini, F, Langeveld JPM, and Groschup MH. Characterisation of atypical BSE cases of the H- and L- type in Germany. Book of abstracts, Prion 2006, International conference on Prion Diseases of NeuroPrion, Network of Excellence, Turin, Italy, 3-6 October 2006.***

After the detection of BSE in the UK in 1986, it was assumed that BSE only occurred as a single strain. However, since 2003 BSE cases of two deviant kinds (L-type and H-type) have been reported in France and Italy in animals that were over eight years of age. Therefore we have re-assessed the 27 German BSE cases that were over eight years of age when the disease was diagnosed out of the 389 German cases that have been detected between November 2000 and December 2005. This characterization focused on the determination of the molecular mass (Mr) of the unglycosylated band as well as on the glycoprofile of the accumulated PrPP^{Sc} by immunoblot analysis. We detected one Ltype case which was characterised by a decreased Mr of the unglycosylated PrP^{Sc} and a unique glycopattern with almost equal percentages of the di- and monoglycosylated PrP^{Sc} P fractions as well as one H-type case with a distinctly elevated Mr molecular mass of this smallest PrPP^{Sc} fraction and an unaltered glycoprofile (using mab L42). Comparative analyses of these two cases together with French and Italian cases of the H- and L-types proved that these two cases indeed belong to the same groups. Both German isolates were inoculated into bovine PrP transgenic mice (Tgbov XV mice). The L-type provoked disease in these mice after very short incubation times of 183 days as compared to 230 days after challenge with classical BSE. In contrast, the H-type has not lead to any clinical symptoms in the mice after 300 days.

Interestingly, the characteristic immunoblot profile of the L-type BSE was maintained after passage through Tgbov XV mice. In order to study the pathogenesis of these novel BSE strains in their natural host, transmission studies in 6 months old calves (i.c. route) have been initiated.

13. **Baron T, Biacabe AG, Bencsik A, Jacobs J, Langeveld JPM , Acutis PL, Polak M, Gavier-Widen D, Buschmann A, Groschup MH and Richt JA. Strain Variability in bovine atypical TSEs. Book of abstracts, Prion 2006, International conference on Prion Diseases of NeuroPrion, Network of Excellence, Turin, Italy, 3-6 October 2006.**

Since 2003, some cases of prion diseases in cattle have shown unusual features as assessed by molecular characterization of the protease-resistant prion protein (PrPres) and/or histopathology, when compared to the unique features of BSE described previously. Similar cases have now been recognized in a number of countries, and an overview of the current situation will be presented. Such studies have allowed to refine the molecular definition of such cases using Western blotting, referred as H-type (Biacabe et al., 2004) or L-type (BASE) (Casalone et al., 2004). While a single strain of infectious agent had previously been recognized when BSE was transmitted to a panel of genetically defined inbred wild-type mice, the recent unusual findings raised the question of transmission of prion disease from such unusual isolates. We could show transmission of prion disease from unusual BSE isolates in murine experimental models (including wild-type and transgenic mouse lines). Most importantly, data obtained during the characterization of experimentally infected mice showed different features when compared to those previously described in mice infected with typical BSE isolates. The unusual PrPres molecular features initially described in the brain of cattle by Western blot were maintained following transmission of the agent into mice. In this presentation, the potential origin of such cases, including the possible existence of "sporadic" forms of prion diseases in cattle, will be discussed.

14. **Hagiwara K, Nakamura Y, Yamakawa Y, Sato Y, Tobiume M and Sata T. Studies on the second atypical BSE case in a Japanese Black Cow. Book of abstracts, Prion 2006, International conference on Prion Diseases of NeuroPrion, Network of Excellence, Turin, Italy, 3-6 October 2006.**

An ELISA (Plateria, BioRad) positive specimen of a 14 year-old Japanese Black cow (beef cattle) slaughtered in an abattoir was

examined by western-blot (WB) and histological/immunohistochemical (IHC) analyses for the confirmation of BSE. Dysstasia had been reported as a clinical symptom. Histological examination of the medulla oblongata at the level of obex showed severe vacuolations in dorsal nucleus of the vagus, nucleus of the solitary tract and nucleus of the spinal tract. Granular and linear deposition of PrP^{Sc} was also detected in these areas by IHC analysis. Thus, histological and IHC data were compatible with the histopathology of the typical BSE. In the WB analysis, however, the amount of the di-glycosylated PK-resistant PrP^{Sc} was found to be at approx. 35% of the total PrP^{Sc}, and the mono-glycosylated PrP^{Sc} was at approx. 40%. The WB analyses showed that PrP^{Sc} distributed widely in the brain with the unchanged glycosylation ratio. Such a glycosylation-ratio is distinct from that of the typical BSE agent in which the di-glycosylated form is dominant (approx. 70%) but, intriguingly, similar to that of the type-2 sporadic CJD agent. No DNA mutation was detected in the PrP coding region, except polymorphisms of the codons for Gln78 and Asn192 being determined as CAG and AAT, respectively. Judging from the glycosylation-ratio, BSE prion herein is different from the typical BSE prion, and the atypical BSE prion found previously in a Holstein steer in Japan. Instead, its molecular feature is close, if not identical, to PrP^{Sc} found in the cattle succumbed to bovine amyloidotic spongiform encephalopathy, and to the sporadic CJD-like PrP^{Sc} in the mice inoculated with BSE agent.



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**ABSTRACTS OF DEPARTMENT FOR ENVIRONMENT, FOOD AND
RURAL AFFAIRS FUNDED RESEARCH RELATED TO BASE**

1. **Project No: SE1795**
Start date: 03. 10. 2005
End date: 02. 10. 2007
Investigation of TSE phenotype variation in British cattle

Evidence suggests that BSE in Britain has presented a constant disease phenotype maintained by a single stable strain of agent throughout the visible epidemic and control measures have been based on this premise. Recently, characterisation of a small number of prion disease cases in cattle in both Europe and Japan has revealed features that differ from this phenotype. This has raised concerns of possible agent strain variation in prion disease of cattle with potential resultant epidemiological impact. However, these studies were limited and the features described are open to a wide range of interpretations, some of which, e.g. host related variables, do not necessarily indicate diversity of the causal agents of TSE in cattle or have consequences for epidemiologically based controls.

Tissue has been collected and stored at VLA - Weybridge from animals identified as clinical suspects throughout the BSE epidemic. This material is a valuable resource and would allow retrospective disease characterisation using, for the majority of cases, pathological approaches that were not available at the time of the original diagnostic examinations of the cases. This will enable the definition of the disease phenotype of BSE, to be extended and will provide the baseline against which unusual features of prion disease in cattle can be compared.

Groups for study will be selected on the basis of confirmed or unconfirmed clinical status and age and examined to establish distribution patterns (profiles) of disease related PrP using

immunohistochemistry on a comprehensive set of brain sections. Where suitable material permits, biochemical characterisation will also be conducted. The approaches used will allow the detection of cases with characteristics suggestive of phenotypic variation whilst consolidating knowledge of the principal phenotype of BSE.

2. **Project No: SE1796**
Start date: 01. 12. 2005
End Date: 01. 12. 2008
Atypical Prion Proteins in cattle

The pathological phenotype of clinical BSE has been remarkably consistent throughout the epidemic in the British Isles. This has greatly facilitated its surveillance and diagnosis. However, about 15% of the clinically suspect animals submitted for diagnosis were negative by examination of the medulla at the level of the obex for standard histological criteria. Significantly, the numbers of these “negative” suspect cases rose and fell with the number of cases of confirmed BSE as the BSE epidemic progressed, a correlation that remains to be fully explained. One possibility is that, like atypical scrapie cases in small ruminants, these “suspects” represent prion-protein-related diseases with a histopathological and biochemical phenotype that differs from ‘classical’ BSE.

We aim to determine whether abnormal PrP is detectable in these cattle and if it represents an atypical form of bovine prion related disease by applying biochemical tests that facilitate recognition of protease sensitive conformers of PrP. Further characterisation of reactors will be undertaken in order to distinguish normal and abnormal phenotypes of PrP in cattle.