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A Decade on from Discovery: The Battle Against Prions

Dr Andrew Bailey at ViruSure GmbH looks at the remaining challenges after 10 years of investigation and regulatory moves to safeguard biotech products from TSE pathogens

Dr Andy Bailey has been actively involved in the pathogen safety of biopharmaceuticals for over 11 years. Originally a Biochemist, Andy served for nine years at the MRC Virology Unit in Glasgow, Scotland. In 1995, he moved as Director of Virus Validation services to Q-One Biotech Ltd, and in 2001 to the Pathogen Safety group of Baxter Healthcare in Vienna, Austria. Over the last 10 years, Andy has presented at numerous regulatory agencies, either in support of products or as an invited speaker at expert workshops, including the UK MHRA, German PEI, French AFFSAPS, US FDA, EMEA and JMHLW (Japan).

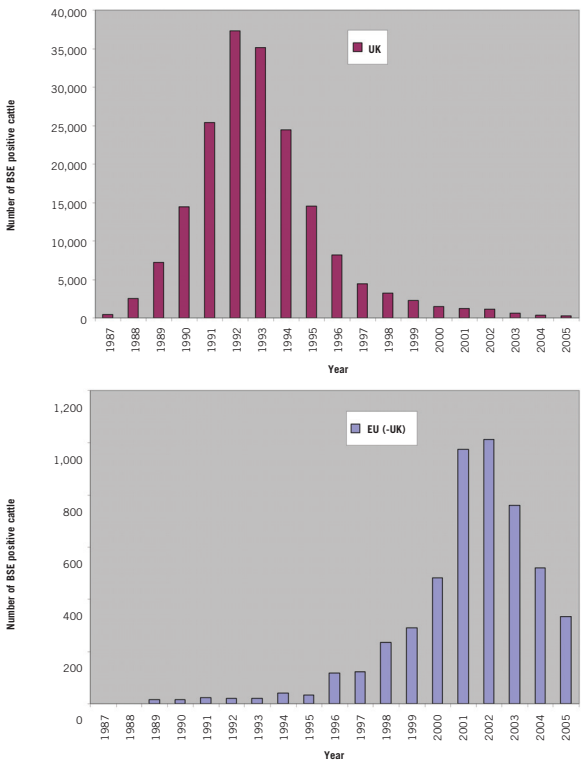
The identification in 1996 of the link between BSE and a previously unseen variant form of Creutzfeldt-Jakob disease (termed vCJD), a transmissible spongiform encephalopathy (TSE) of man (1), triggered a sequence of revolutions in the regulatory framework for TSEs and the safety of biopharmaceutical products. The biopharmaceutical industry was faced with regulating an infectious agent for which, even today, many unknowns still exist and which had already peaked (at least for BSE in the UK) and which evaded many classical means of eradication. This article takes a look at how far we have come since 1996 and the potential issues still confronting the biopharmaceutical industry in relation to prions.

THE BSE LEGACY – WHERE ARE WE TODAY?

It was recognised as early as 1988 that contaminated meat and bonemeal (MBM) was the most likely route of transmission, and the 1988 UK MBM feed ban was largely successful in stemming the spread of BSE, even though the long incubation period (around five years) meant that cases continued to increase until 1992/93 – peaking at around 35,000 cases per year (detected only by passive surveillance, see Figure 1). Comparable MBM bans in Europe and the US were only implemented in 1994 and 1997 respectively but, as in the UK, these measures came too late to prevent an increase in BSE cases.

When viewed as a whole, numbers of BSE cases within the EU core member states (UK excluded) peaked in 2002, although in comparison with the UK, the prevalence of BSE in mainland Europe is around 10- to 100-fold lower. Differences in peak BSE incidence do exist between the individual states, with peaks occurring between 1999 and 2003. These differences most likely reflect the effectiveness of implementation of the 1994 MBM ban. Implementation of the feed ban was even slower in some of the new acceding EU member states (Poland and Czech Republic, for example) which have only recently been subject to EU

Figure 1: Number of BSE positive cattle detected in the UK or in the rest of the EU (UK excluded)



controls and, in contrast to the rest of Europe, BSE prevalence continued to rise in these countries during 2005 (2). The late implementation of a MBM feed ban in the US has likewise resulted in three BSE cases in US cattle (one imported from Canada), but comparisons of BSE prevalence in the US and EU are difficult due to the lower level of surveillance carried out within the US. Surveillance efforts in the US will be further curtailed in future years as post-mortem BSE testing has been suspended (3).

BSE AND BIOPHARMACEUTICALS REGULATION

When the link between BSE and vCJD was established, the EU moved immediately to try and stem the threat to human health through Directive 97/534/EC, which prohibited the use of specified risk materials (SRMs) in bovine-derived substances. However, implementation of this directive immediately ran into problems as it effectively eliminated a large proportion of pharmaceuticals from the market. The implementation of Directive 97/534/EC was postponed on several occasions, and was eventually superseded in 2000 by Directive 2000/418/EC, which delegated the regulation of the bovine components used for the production of medicinal products to the EMEA. For medical devices, which do not fall under the remit of the EMEA, the later Directive 2003/32/EC further established a framework for BSE regulation.

The EMEA Note for Guidance (EMEA/410/01) on minimising the transmission of TSE agents through human and veterinary medicinal products established BSE risk management procedures which have since become the foundation for TSE risk management worldwide. This risk management philosophy developed to a large extent out of the geographical BSE risk (GBR) system established by the EU Scientific Steering Committee (SSC), which emphasised the importance of sourcing and ensuring the absence of SRMs from all production systems. The important aspects of BSE risk management as detailed in EMEA/410/01, include:

- Country of origin
- Feedstuffs during rearing
- Herd certification and traceability
- BSE monitoring (including post-mortem testing)
- Stunning procedure
- Removal of SRMs (and segregation of processes for removal of SRM)
- Sanitisation of areas and equipment used
- Stringency of veterinary inspections at the slaughter house
- Potential TSE removal via the manufacturing process

The distribution of vCJD cases

UK, 164; France, 21; Republic of Ireland, 4; Italy, 1; USA, 3 (two long-term residents in the UK and one a former resident of Saudi Arabia); Canada, 1; Saudi Arabia, 1; Japan, 1; Netherlands, 2; Portugal, 1; and Spain, 1.

The task of ensuring compliance with these guidelines rests with the manufacturers of the medicinal products. By far the easiest way of demonstrating this is through the use of bovine materials that have been certified by the European Directorate for the Quality of Medicines (EDQM), which offers a certification process for compliance with these risk management principles.

THE vCJD REGULATORY FRAMEWORK – PRECAUTIONARY PRINCIPLES

The risk strategies for management of the various human forms of CJD differ markedly. For the sporadic form (sCJD), documented iatrogenic transmissions have been observed via surgical procedures, human cadaveric pituitary-derived growth hormone and corneal transplants. There have been no documented or identifiable transmissions, however, through blood and blood components or human plasma-derived products, despite the use of product manufactured from blood or plasma pools to which donors who later developed sCJD contributed (4). It is primarily for this reason that neither the EU nor the US requires the recall of manufactured plasma products for sCJD.

In contrast to sCJD, vCJD demonstrates a different aetiology, with a higher concentration of the disease marker found in lymphoreticular tissues, and by implication higher levels are likely to be found in blood. Furthermore, the age distribution for vCJD occurs in a younger age group (that is, those more likely to donate blood and/or plasma). A problem encountered in the early days of setting regulatory policy with respect to vCJD was the lack of knowledge around whether the disease was transmissible through blood. Animal studies supported the notion that blood from preclinical vCJD donors would be infectious (5), although the applicability of such data to vCJD was strongly debated. From the outset, therefore, the precautionary principle (action based on scientific proof to follow) has guided regulatory policy for managing vCJD risk. With three probable vCJD transmission events within the UK to date (6,7,8), the burden of scientific proof has to a large extent been fulfilled, although question marks still surround the distribution of infectivity within blood, blood components and plasma.

To date, the only risk factor considered a prime determinant for the potential to contract vCJD has been exposure to BSE contaminated foodstuffs, which by default is linked to residence in a high risk country. The worldwide incidence of vCJD on the whole supports the premise that residency in a high risk BSE country as the primary determinant for vCJD risk. However, there has been one case in Japan and two cases in Saudi Arabia (countries with little or no reported cases of BSE) involving individuals with little or no identifiable residence in high risk countries. This highlights the difficulty in identifying where exposure to BSE has occurred. Until 2004, vCJD was restricted largely to the UK or former residents of the UK, a few cases in France, and one in Italy. As of 1st December 2006, a total of 199

clinical vCJD cases have been reported worldwide, of which 197 have been attributed to dietary exposure to BSE, and two to secondary transmission via blood products. All of the clinical cases to date have been MM homozygous at codon 129, and only one transfusion-transmitted case who did not have overt clinical disease was MV heterozygous.

Considering the nature of the risk, regulatory policy for managing vCJD risk has focused on two main areas. First, the deferral of donors based on residency in a high risk area and secondly, potential prion removal by the manufacturing process.

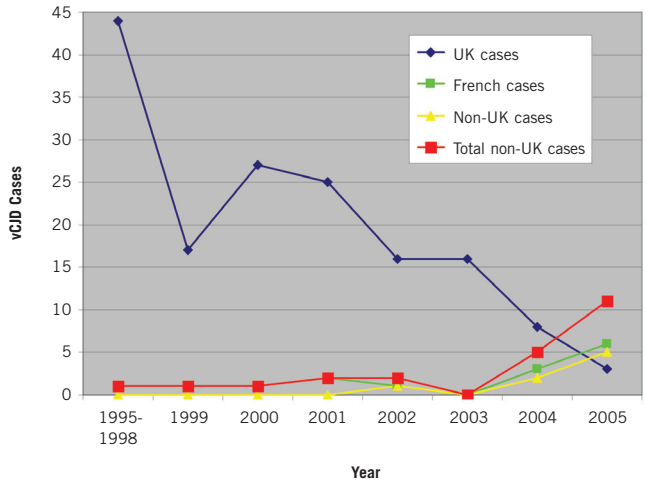
Current deferral policies established by the US FDA require the deferral of donors who have resided cumulatively for more than three months in the UK from 1980-1996 (in the EU, deferral is set at more than one year's residence in the UK), with an additional deferral of donors who had lived for more than five years in France between 1980 and the present. The FDA has also implemented donor deferral for more than five years residence in Europe between 1980 and the present for blood products and components (that is, those products where potential removal via the manufacturing process is unlikely). The exclusion of plasma-derived medicinal products from the latter deferral criteria was based largely on the body of positive data that had been presented by the plasma products industry demonstrating the ability of manufacturing processes to remove potential prion contamination.

The EMEA has established guidelines for the investigation of prion removal via manufacturing processes (9), and such studies are now mandatory for purified plasma products. However, these guidelines stop short of standardising either the spike form or type of assay. New assays for the detection of TSEs are in development that may supersede existing assays either in terms of sensitivity or functionality, and standardisation of assay in the current climate would stifle necessary research. Likewise, standardisation of spike forms at the present time presumes a level of understanding of the nature of infectivity that has not yet been reached. As long as the infectivity in blood continues to resist attempts at biochemical characterisation, this will remain the case. There is a general agreement, however, between regulatory bodies that manufacturing processes for such products provide for significant removal of potential prion contamination, although debate as to what level of removal should be considered sufficient to ensure safety is ongoing.

SCREENING FOR TSEs IN BLOOD – THE ETHICAL DILEMMA

Largely because of the potential threat of vCJD transmission through blood, the last 10 years has witnessed significant time and effort invested in the search for a sufficiently sensitive and validatable assay able of detecting prion infectivity blood or plasma (10). Almost all assay developments have focused on detecting the infectious marker found in brain (in other words the protease resistant form of the prion protein) on the assumption that this is the form likely to be encountered in blood. However, the challenge of detecting an infectious agent in blood/plasma, which to date has yet to yield to biochemical characterisation, are not to be underestimated. Extreme care is also needed in the implementation of any test that has even a small percentage of false positives. In the EU alone, more than 10 million units of blood are donated annually, and even a small percentage of false positives, particularly without a secondary confirmation test, would give rise to an unacceptably high number of false positive individuals, with the ensuing major ethical issues. Therefore steps toward the implementation of any diagnostic screening test need to be carefully planned and any such test must be subject to independent scrutiny using reference prion materials.

Figure 2: Number of vCJD cases (by date of diagnosis) through 2005 in the UK, France and other countries



BSE & vCJD – CURRENT PERSPECTIVES

The mature and extensive BSE legislation within Europe reflects the importance attributed to this subject over the last 10 to 15 years. But the management of risk however comes with a significant cost burden. The EU wide cost of BSE monitoring from 2001-2004 is estimated to be around €1.8 billion, and the cost of identifying a single BSE infected animal in the age group more than 48 months has been estimated at €64 million (11). With the declining incidence of BSE within the EU, the commission has begun the process of identifying the conditions that need to be met in order to allow any relaxation in BSE legislation, whilst maintaining consumer confidence and safety. Given the political turmoil that ensued following first the UK BSE crisis, and subsequently the EU BSE crisis, any reduction in control measures, however small, will require the full courage of the member states to pull back from what is a mature and effective legislative framework. Emerging pathogens place a burden on available finances and resources that can be dedicated to fighting zoonotic agents. The trend is ever towards a rationalisation of available resources, and the

management of such resources is increasingly being driven through science-based risk management. Such risk management cannot ignore the decreasing trend in BSE cases within Europe, and opens the door to reducing the economic burden imposed by BSE within the EU. However, it is clear in some of the acceding EU lands that tighter controls will need to remain in place given that the prevalence of BSE in these countries is still increasing.

Figure 2 depicts the vCJD incidence trends for the UK, France and for cases not believed to be the result of BSE exposure within the UK or France. The trend towards a higher number of vCJD cases in non-UK residents (mainly residents of mainland EU), most likely reflects the later peak exposure to BSE in Europe and other areas of the world. With an increasing number of vCJD cases within mainland Europe, the probability of finding vCJD plasma or blood donors who have contributed to plasma pools used for the manufacture of plasma-derived medicinal products is also increased (donor exclusion for residency in Europe is implemented in only a few countries). At least for human plasma-derived products, the removal afforded through the manufacturing process is likely to provide significant assurances should such an event occur. That said, a strong debate continues in relation to what level of removal should be considered sufficient to assure safety with respect to vCJD, and presents the regulating authorities with a complex issue for which guidance would be advantageous.

While all the clinical cases of vCJD so far have been homozygous for methionine at codon 129 (MM), the detection of the prion marker in one transfusion related case who was MV heterozygous suggests that this genotype may also be susceptible. Data from animal studies would support the notion that MV and VV genotypes would be less susceptible to transmission than MM, but these studies have also identified sub-clinical infections carrying the disease marker in MV and VV genotypes (12). The results of the UK study evaluating anonymised appendix/tonsil specimens showed that vCJD infection might be more common than is suggested by the number of vCJD to date (13). Furthermore, two of the three positive samples could be confirmed to be of the VV genotype. The risks that such a sub-clinical population might pose to the blood products industry is difficult to evaluate. Currently the UK is extending this study to examine a larger number of specimens in order to more precisely define the prevalence of supposed sub-clinical carriers.

In conclusion, the regulatory framework for the management of TSE risk has matured greatly since 1996 and has spurred research that has significantly advanced our understanding of prions and prion risks. No significant changes to this regulatory framework have occurred in the last two years and unless a new risk profile emerges it is likely that this will remain the case. As always, however, the pharmaceutical industry must remain alert to developments in this field that might warrant a potential shift in regulatory policy. ◆

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