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A BRIEF OVERVIEW OF BOVINE SPONGIFORM ENCEPHALOPATHY AND RELATED DISEASES INCLUDING A TSE RISK ANALYSIS OF BOVINE STARTING MATERIALS USED DURING THE MANUFACTURE OF VACCINES FOR USE IN HUMANS

KRÓTKI PRZEGLĄD WIEDZY O ENCEFALOPATII GĄBCZASTEJ BYDŁA I PODOBNYCH CHOROBACH Z UWZGLĘDNIENIEM RYZYKA ZASTOSOWANIA SUROWCÓW ZWIERZĘCYCH W PRODUKCJI SZCZEPIONEK DLA LUDZI[1]

Praca zawiera zarys podstawowych informacji klinicznych dotyczących encefalopatii gąbczastych u zwierząt ze szczególnym uwzględnieniem encefalopatii gąbczastej bydła - BSE. Autor przedstawia współczesne poglądy na szerzenie się tych chorób, ich przenoszenie drogą pokarmową lub parenteralną za pośrednictwem białek pńonowych (PrP). Podaje informacje o warunkach w jakich powstaje zagrożenie dla populacji ludzkich oraz sposobach zabezpieczania się przed tymi zagrożeniami przez wprowadzanie odpowiednich restrykcji w zakresie hodowli, a przede wszystkim karmienia bydła i utylizacji odpadów zwierzęcych. Analizuje ryzyko dopuszczania mięsa wołowego i produktów zwierzęcych do spożycia oraz wykorzystania ich w przemyśle farmaceutycznym - zwłaszcza przy produkcji szczepionek - i w przemyśle kosmetycznym. W analizie uwzględniono zakaźność różnych tkanek i prionów oraz dróg ich przenoszenia międzygatunkowego, szczególnie ze zwierząt na ludzi.

INTRODUCTION

Had this conference on zoonoses been held in 1984, this paper would not have been presented. This is because until this time, none of the three animal transmissible spongiform encephalopathies (TSE) that had been described, (scrapie of sheep and goats, transmissible mink encephalopathy (TME) of farmed mink in North America and Northern continental Europe, and chronic wasting disease (CWD) of North American deer and elk) was regarded as a zoonosis. In particular, no TSE or prion disease had been reported in cattle anywhere in the world. This is still the case for pigs and poultry. These species currently do not present a TSE risk.

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[1] Praca przedstawiona na Konferencji "NOWO POJAWIAJĄCE SIĘ, NAWRACAJĄCE I BIOTERRORYSTYCZNE ZAGROŻENIA ZE STRONY ZAKAŹNYCH CHORÓB ODZWIERZĘCYCH", 9 maja 2001 r. w Warszawie.

Evidence to support the absence of a connection between scrapie in sheep and human Creutzfeldt-Jakob disease (CJD), a human TSE, comes from epidemiological studies conducted worldwide, but mainly in France where both scrapie and CJD occur (1, 2, 3). Important evidence comes from Australia where no animal TSE exists, but where there is a similar incidence of CJD in humans to that occurring in all other parts of the world, namely 1-2 cases per million per annum.

This encouraging state affairs was to change dramatically with the announcement, on 20 March 1996, of ten cases of a new variant form of CJD (vCJD) in the United Kingdom (UK) (4). This followed the identification of bovine spongiform encephalopathy (BSE) in 1986 in the UK (5) and its subsequent development (6). BSE is a fatal, progressive, neurological disease of domestic cattle used to produce milk and meat for human consumption. At the present time (May 2001) 95 definite or probable cases of vCJD have been reported in the UK *(85,* + 6 alive, + 4 with pathology pending, Department of Health Press Release), 3 in France and 1 in the Republic of Ireland, all countries with BSE in native-born cattle. There have been serious consequences for animal health, public health and trade in cattle and cattle products in affected countries. The World Health Organisation (WHO), the Office International des Epizooties (OIE) (7), the European Commission (EC) and governments worldwide have addressed the issues and produced useful guidelines, made recommendations and introduced measures respectively. These are aimed at protecting public and animal health and eliminating the disease in cattle. The UK has had by far the largest epidemic (approaching 180,000 confirmed cases) but cases have been reported in native-born cattle in twelve other countries of Western Europe. Stringent enforcement of the appropriate measures in the UK and Switzerland (378 confirmed cases) has resulted in decline towards elimination. The epidemics in other countries are not certainly falling or are minuscule.

From an early stage in the BSE epidemic, clinically affected cattle have been compulsorily slaughtered and totally destroyed, thus eliminating risk. However, as the mean incubation period for BSE is 60 months and since there is no test to detect infected live animals there is a risk from unidentifiable, infected cattle that are slaughtered for human consumption, killed for other reasons or die. To reduce any TSE risk, potentially infected tissues (specified risk materials or SRM) are removed from all at risk cattle in the European Union (EU) and Switzerland. These tissues comprise at least bovine central nervous system (CNS) tissue and intestine, the only tissues in which BSE infectivity has been detected.

Cattle are also the main source of animal materials used in the manufacture of medicinal products. Concerns have been expressed because of the possible contamination of biological products, including vaccines and other medicinal products and devices, as a result of the use of during manufacture of bovine material that might be infected with the BSE agent. Part of this paper deals with the assessment of this risk and how it is effectively managed so as to maintain the safety and public confidence in vaccines for human use, including in children. These features are of the utmost importance to maintain the current level of protection against serious, contagious, and sometimes fatal, diseases.

TSE AND PRION DISEASES

Scrapie of sheep has been known since at least the early XVIIIth century and is found occasionally in goats and rarely in moufflon. CJD was not discovered until the early 1920s but probably existed before. Subsequently a very rare familial form of human TSE called Gerstmann-Straussler-Scheinker disease (GSS) and a geographically localised epidemic of a human TSE called kuru in Papua New Guinea, transmitted at funeral ceremonies in which the whole human body was consumed, were described. All have been demonstrated to be experimentally transmissible even though GSS and familial forms of CJD are inherited. Originally this group of diseases was called the sub-acute, transmissible spongiform encephalopathies. In the UK and in some other countries, TSE have been reported in captive wild BOVIDAE and FELIDAE and in domestic cats (8). All these diseases are related to BSE and are presumed, or confirmed, to have been caused by the BSE agent.

In 1982 (9) first reported the identification of a small proteinaceous infectious particle or prion comprised of a partially protease resistant, host protein (subsequently named prion protein or PrP) that was considered alone to be responsible for scrapie and other TSE infections. Thus the prion theory of TSE agent structure was born (for other hypotheses and further information see Schreuder, (10) and Aguzzi and Heppner (11)). Because PrP is found in the brain of virtually all individuals affected with TSE, these diseases are also called prion diseases.

Identification of the PrP gene led to the identification of various mutations in humans that were responsible for about 10-15% of cases of CJD with a familial origin, so-called familial CJD. Mutations leading to spontaneous disease in animals have not been found but polymorphisms in this gene in humans and in sheep appear to significantly influence the length of the incubation period in natural disease.

Iatrogenic transmission of CJD in man has occurred in the past as a rare event but never through the use of vaccines or any animal product. However, accidental transmission of scrapie to sheep or goats has been reported twice but never with the use of commercially produced vaccines and bovine material was not incriminated.

There have been no known incidents of CJD or vCJD being caused by the use of vaccines. Nevertheless the potential risk exists, but only if one or more starting materials contains infectivity. This paper provides the guidance necessary to ensure that any risks are eliminated or negligible.

Prion diseases are all neurological diseases, have long incubation periods often measured in years or decades in humans, and in years in animals. They are invariably fatal. The agents that cause them vary in their molecular (12) and biological properties (13) leading to the identification of molecular and biological strains. How these are related to each other still needs clarification. All TSE agents are resistant to destruction though autoclaving in 2M sodium hydroxide (14) and treatment with sodium hypochlorite providing 20,000 ppm of available chlorine appear to demolish detectable infectivity (15). The agents resist destruction following burial, treatment with formaldehyde, exposure to ultra-violet and ionising radiation and dry heat up to 600°C (16).

Tests for the presence of disease-specific PrP (PrP-res) in contrast to the normal cellular form (PrP-sen) are now widely used to confirm disease by immunohistochemistry, immunoblotting and a range of other methods including the detection of

aggregated fibrils (so-called scrapie-associated fibrils or SAF) by negative stain, electron microscopy.

The only tests for infectivity are bioassays in experimental animals. These are impractical for routine use, but they enable the identification of biological strains of agent when multiple strains of inbred laboratory mice are used. In this way the single major strain of agent from cattle with BSE and related diseases can be distinguished from most forms of CJD and all strains of scrapie (13). The length of the incubation period and the lesion profile within the brain are the criteria used to distinguish strains. In this way it has been possible to confirm the similarity of strains in isolates from cases of BSE in cattle and vCJD in human patients thus strongly supporting the notion that the BSE agent is responsible for all cases of vCJD so far identified, but not for any of the historical forms of CJD nor of scrapie (17). Rapid PrP tests (that do not determine the presence of infectivity) have been developed and evaluated (18) and are now in use for surveillance of BSE and other purposes in the EU.

ORIGIN OF BSE AND vCJD

According to Wilesmith et al (19) and Wilesmith, Ryan and Atkinson (20) the origin of BSE is from a scrapie-like agent from sheep, or a cattle-adapted scrapie-like agent from cattle. The vehicle is meat-and-bone-meal (MBM) produced mainly from animal waste from abattoirs and butcheries, by rendering processes that did not sufficiently inactivate TSE agents in the raw material. Wilesmith, Ryan and Atkinson (20) showed that a significant reduction in the amount of MBM produced by the hydrocarbon solvent extraction of tallow in the period 1981-1982 (the presumed earliest dates for effective exposure of cattle) may have been responsible for allowing sufficient TSE infectivity to pass into the MBM to cause clinical disease from 1985/6 after the incubation period was complete. However the BSE Inquiry Report (21) suggests an alternative origin from a PrP gene mutation in cattle or other species in the 1970s, followed by recycling via MBM. The BSE Inquiry further states that in their view no rendering system in place before 1988 was effective at completely destroying TSE agent infectivity.

It seems most likely that cattle, captive wild BOVIDAE and perhaps domestic cats were infected, like cattle, via MBM. Captive wild FELIDAE were more likely infected by consumption of uncooked cattle heads and vertebral column containing infected CNS material from cattle.

MEASURES TO CONTROL BSE AND PROTECT PUBLIC HEALTH

The main measure to protect animal health is some form of ban prohibiting the use of ruminant or mammalian protein or MBM to be fed to ruminant species. In the UK it was prohibited from July 1988 to feed ruminant protein to ruminant animals. Exceptions were made for milk, which is not infectious, and some other products. This ban was very effective, as revealed by the effect on the epidemic curve (Figure 1). It reduced the hazard of developing disease in the succeeding twelve months by 67% (Stevenson et al, 2000). Because no country has been able to prevent completely the occurrence of BSE in animals born after the date of the feed ban in their country, an EU-wide measure has been introduced to prohibit the feeding of mammalian protein

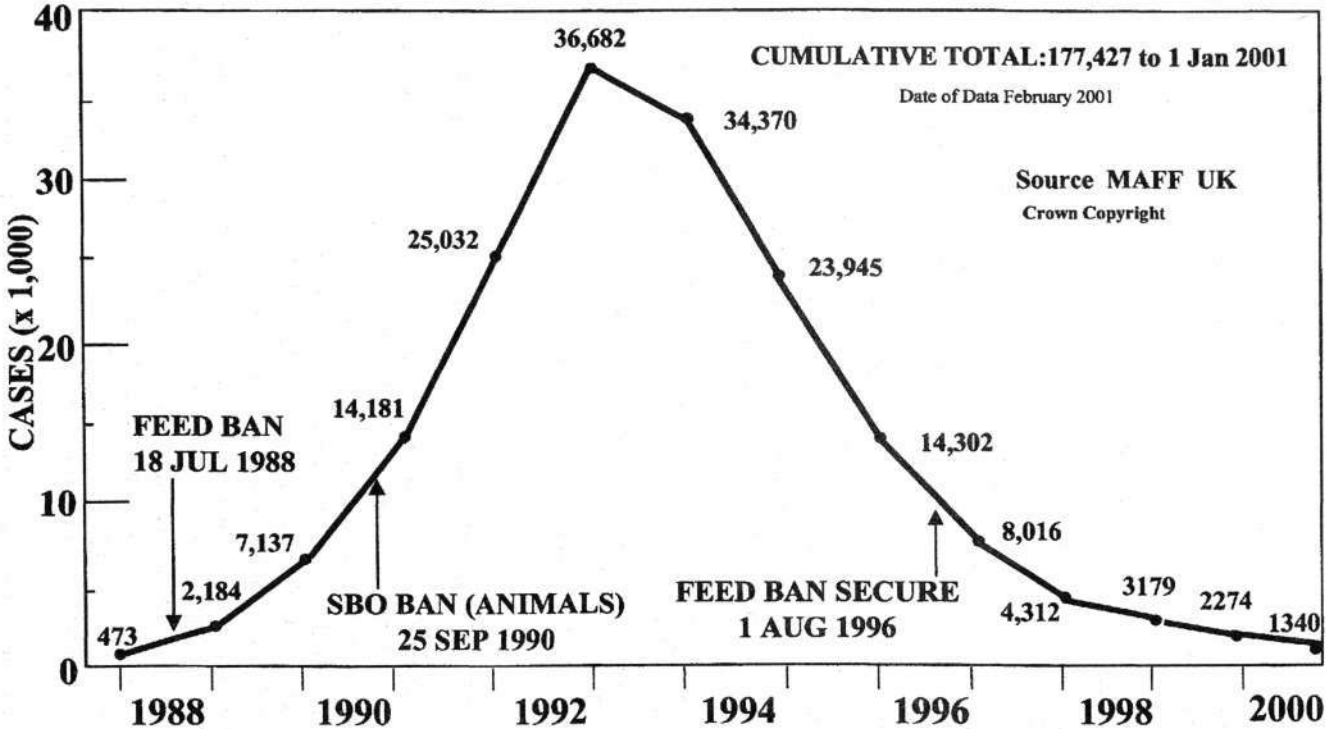

Fig. 1. Number of confirmed cases of BSR by year in GB

(with some exceptions, like milk) to any food animal species from 1 January 2001. The UK had a similar ban introduced from March 1996 that appears to have been responsible for the continued decline of the cattle epidemic (Figure 1) and has restricted the occurrence of BSE in cattle born after the effective date of the ban (1 August 1996) to just one animal.

From 1988 in the UK, and from 1990 in the EU, all cattle suspected to have BSE have been compulsorily slaughtered and destroyed, thus very significantly reducing any human health risk from the most highly infected animals and their tissues, and preventing recycling of infection back to cattle via the rendering system, MBM and cattle feed. However, this only partially dealt with the risk since it did not cover that from clinically healthy infected animals for an average period of five years after exposure and before clinical signs were evident. This risk is dealt with by another ban, initially a specified bovine offals (SBO) and subsequently a specified risk materials (SRM) ban.

From 1989 in the UK, from 1990 in Switzerland, but not until 1 October 2000 throughout the EU, a SRM ban has been introduced. This ban identified the tissues from cattle and sheep that would be likely to contain any, (cattle) or high levels of TSE infectivity (sheep) during specified periods of incubation. These include the CNS and skulls of both species (because eyes, cranial ganglia and brain in which infectivity can occur cannot be completely removed from the skull) and the spleen of sheep and goats. BSE infectivity has been found in the spleen of sheep and goats experimentally, orally challenged with 0.5g of BSE-infected bovine brain. BSE has not been identified as a natural disease of sheep or goats. The ban as applied to sheep and goats is therefore part of a risk reduction policy to protect all species from exposure to high levels of scrapie infectivity (that exists now) and BSE infectivity should it arise in the future.

IMPORTANCE OF BSE FOR THE PHARMACEUTICAL INDUSTRY

More than 80% of all pharmaceuticals (including biologicals such as vaccines) contain materials of bovine origin. It is therefore important to establish that any Starting materials derived from cattle (and other animals susceptible to TSE) are devoid of TSE infectivity or that any risk is appropriately managed. Animal materials for use in the manufacture of pharmaceuticals are derived only from animals bred, born, raised, killed and passed fit for human consumption. This is because such animals are under veterinary supervision, their source and disease status is known and believed to be compatible with safety in regard to a range of infectious diseases (including zoonoses) toxins, antibiotics and other residue hazards. Of the common food animal species used as a source of animal material for pharmaceutical manufacture only ruminant species (cattle, sheep and goats) are known to be susceptible to TSE. Table I lists materials of ruminant origin potentially used in vaccine manufacture from 1980 to date.

If it were possible to test each batch of starting material for infectivity, then there would be no problem. Unfortunately, the only way to detect infectivity is by bioassay and this is impractical because it takes too long (years), is expensive and is ethically undesirable, since animals have to be used. The alternative, using the detection of

PrP-res as a proxy for infectivity is also unacceptable despite it being a rapid and less expensive method. This is because current tests are far less sensitive than bioassay (by about 1000 times) and a negative result cannot be equated with absence of infectivity. Thus the way forward has been to conduct thorough risk analyses and develop risk management strategies that eliminate or reduce any risks to negligible levels bearing in mind that it is never possible to prove a zero risk.

TSE RISK ANALYSIS

Since the advent of BSE in 1986, the biologicals and pharmaceutical industries have done a great deal to develop their safety protocols, to change the source of certain starting materials that might theoretically present a TSE risk and at all times to liase with regulatory bodies to ensure that the actions taken or proposed are acceptable and safe. An on-going, independent and generic risk analysis for all starting materials has been conducted by SmithKline Beecham Biologicals, (SBB), (now GlaxoSmithKline, (GSK)) in order to review and ensure the safety of all final products they produce.

In order to conduct the analysis, full information was provided by the Company on the type of starting material, its precise origin and use. In this context it is important to note that the animal origin starting material purchased by the Company might not be the starting material and thus the primary source for the supplier. For example, amino acids might be a starting material for the biological company but the animal origin of this might have been bovine bones. Therefore the risk analysis would be initiated on bovine bones and the processes used to generate the amino acids, not on the amino acids themselves.

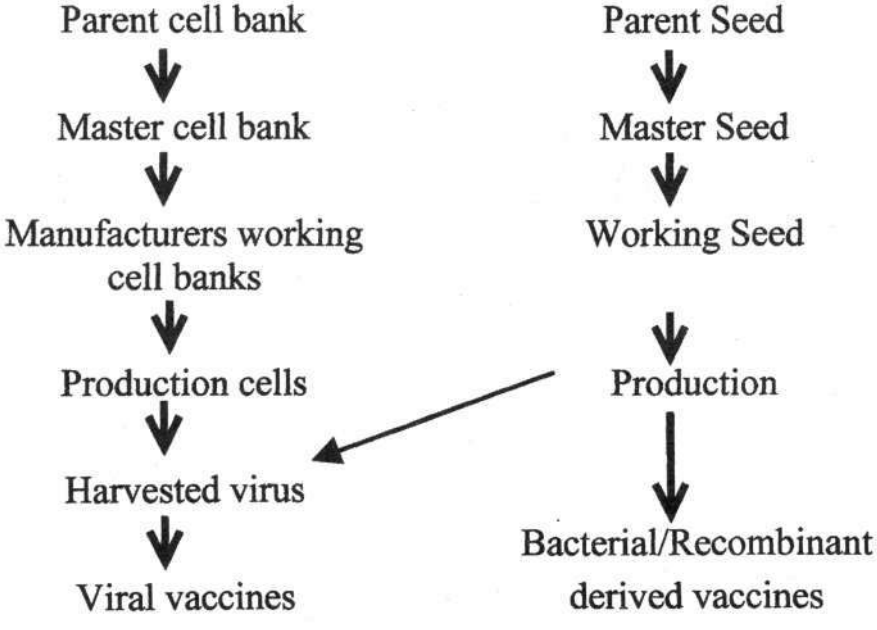

Fig. 2. The seed lot principle

Ta b 1 e I. Materials of ruminant origin used in vaccine manufacture since the 1980s

WHO/CPMP Category	Tissue/Full name		Purpose	Vaccine
IV	Milk derived products	Lactose	Stabiliser freeze drying, Medium pre-culture	MMR, Hib, Men
		Skimmed milk	Stabiliser for seeds	DTP vaccines
		Casamino acids	Growth media, storage media	DTP vaccines, Hib
		Casein peptone	Growth media	DTP vaccines, Men, Typh
		Lactalbumin hydrolysate	Growth media	Polio
		Galactose	Growth media	MMR, HAV
		Hycase/Casein hydrolysate	Growth media	Hib
		Casein	Growth media	Men
IV	Muscle extracts (including heart)		Bacterial growth media	DTP vaccines, Men, Typh, Hib
IV	Blood products	Fetal Calf serum	Cell growth and virus replication	HAV, MMR
		Donor Calf Serum	Cell growth and virus replication	Polio
		Haemoglobin/Hematin	Bacterial growth media	Hib
		Sheep blood	Growth media for Pa Master Seed	DTP vaccines
IV	Bile	Sodium deoxycholate	Detergent	Flu
		Choline chloride	Viral growth (Medium 199)	All viral vaccines
IV	Hides	Hydroxy-L-proline	Growth media	MMR
IV	Wool (lanoline)	Cholesterol	Viral growth media (Medium 199)	All viral vaccines
III	Pancreatic enzymes	Meat digest media	Growth media	DTP vaccines
II	Spleen	Spleen derivative	D Master Seed culture	DTP vaccines
		(+ Cat. IV)		
Not categorised (NC)				
NC	Gelatin derived from	Gelatine derivatives	D Master Seed stabiliser	DTP vaccines
	bovine bone	Amino acids	Growth media	Most vaccines
NC	Tallow derivatives	Glycerol	Seed stabiliser	Hep B, Hib, Men, Typh, DTP
		Tween 80	Emulsifier	MMR, Polio

There are three important points to make in regard to the risk analysis. First, that there is a wide range of starting materials (Table I). Second, the seed lot principle of vaccine manufacture (Figure 2) introduces an important historical dimension. Third, risk analysis is a dynamic process, since changes in knowledge and legislation significantly affect the outcome over a period of time.

A TSE risk analysis takes account of three main factors: the SOURCE (geographical, species and tissue), PROCESS and USE. It is important to note that there are temporal changes in risk such as the occurrence of BSE in a country previously believed to be free of the disease. An example is the recent occurrence of BSE in Denmark, Germany, Italy and Spain some 14 years after its discovery in the UK. There has also been new information such as on the distribution and timing of BSE-infectivity in the tissues of cattle, and new legislation. Furthermore the knowledge of the provenance of starting materials has improved with time. What was acceptable in 1986 is no longer acceptable.

The risk analysis identified the original animal and tissue source of the starting material. The TSE risk in this material was assessed using the latest scientific data. The impact of any processing was also assessed.

THE SEED LOT PRINCIPLE

In regard to the seed lot principle (Figure 2), Master cell banks (for viral vaccines) and Master seeds for bacterial and viral vaccines, may have been produced before BSE had been discovered. According to the seed lot principle, Working and Production cells and seeds come downstream of the Master cell banks and seeds. They can be much more readily re-established than the Master seeds and banks from which they are derived. No risk been identified that is higher than negligible or remote in Master cell banks and Master seeds.

In regard to the Working and Production cell banks and seeds, in no case has a TSE risk been identified that is higher than negligible or remote, either. Nevertheless, as a result of the Company adopting a precautionary principle, re-derived Working cells and Working seeds have been, or are being, produced. Ruminant sources have been avoided where possible or new starting materials are used with a specification that reflects the state of the art.

Changing Master cell banks and Master seeds would inevitably result in disruption of vaccine supplies and thus leave some patients without protection from contagious and fatal diseases. Since no risk greater than negligible or remote has been identified in the starting materials, Master cell banks and Master seeds have been maintained unaltered. Major regulatory bodies have endorsed this approach and thus no disruption to the vaccine supply has occurred and patients remain just as well protected as before.

GEOGRAPHICAL RISKS

Geographical risks can only be assessed when adequate data are supplied and surveillance for BSE and other human and animal TSE is adequate. It is insufficient to just rely upon reports of BSE occurrence though detection of the disease does indicate an awareness of the problem. Three countries without reported BSE in native-born cattle have reported the disease in small numbers of cattle imported from

countries with BSE. These are Canada (one case), the Falkland Islands (one case) and the Sultanate of Oman (two cases). These incidents are of little consequence so long as they are detected, confirmed by laboratory examination and the carcase is totally destroyed.

Ta b 1 e II. Countries with BSE in native born cattle

GREAT BRITAIN	177,857	
NORTHERN IRELAND	1,812	
REPUBLIC OF IRELAND	587	(12)
SWITZERLAND	378	
FRANCE	272	(1)
PORTUGAL	558	(6)
THE NETHERLANDS	14	
BELGIUM	28	
LUXEMBOURG	1	
LIECHTENSTEIN	2	
DENMARK	2	(1)
GERMANY	55	(6)
ITALY	1	(1)
SPAIN	43	

( ) additional number of imported cases
Data from MAFF/DARD/OIE/EC to various dates in 2001

BSE in native-born cattle has only been reported in 13 countries of Western Europe (Table II). The UK has by far the largest epidemic and over 40,000 of these cases have occurred in cattle born after the introduction of the feed ban in 1988. But only one case has occurred since 1 August 1996, the date on which a ban on feeding mammalian protein to all food animal species in the UK was declared effective. The epidemics in the UK and in Switzerland are declining in response to the various control measures (Figure 3). In some other countries the epidemics are still rising (Figure 3) and in others there are too few cases to make a judgement (Table II). The use of PrP testing of targeted populations of cattle in some countries (e.g. Switzerland, France and Germany) as part of an active surveillance or research programme has revealed cases of disease in animals without reported clinical signs. Most BSE cases now occurring in all these countries are in cattle born after the date of introduction of the feed ban in the respective country. New EC legislation introduced in 2000 and 2001 on SRM and feed (similar to the measures in the UK) should result in all epidemics declining to obscurity if completely enforced, but this may take some years yet.

BSE has been introduced into countries by other means than imported animals. Imported MBM (Figure 4) constitutes the major risk Apart from these exogenous risks there are also endogenous risks generated from TSE in any species being recycled through MBM feeding, after ineffective rendering. It is clear that the precise geographical destination of cattle and mammalian MBM exported from countries with BSE is uncertain (Figure 4). Therefore, the analysis of the risk of TSE infectivity by

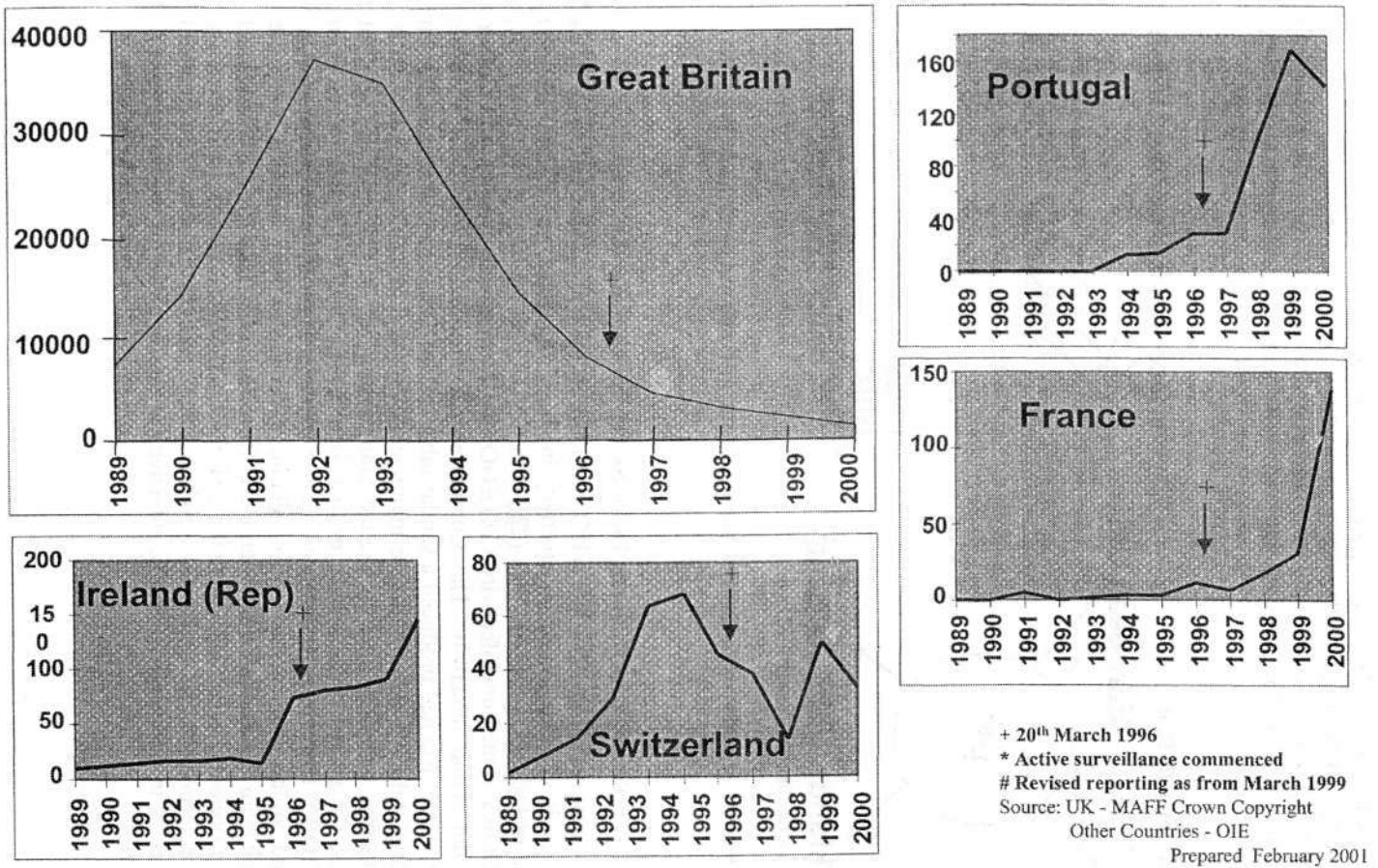

FIGURE 3

EPIDEMIC CURVES BY COUNTRY

Fig. 3. Epidemic curves by country

type of tissue is of fundamental importance. Reliance should not be placed entirely on the geographical origin of the imported material.

On a more positive note it can be stated that the safety of the final (biological) product, in regard to TSE risks, is largely and reliably determined by the safety of the starting material.

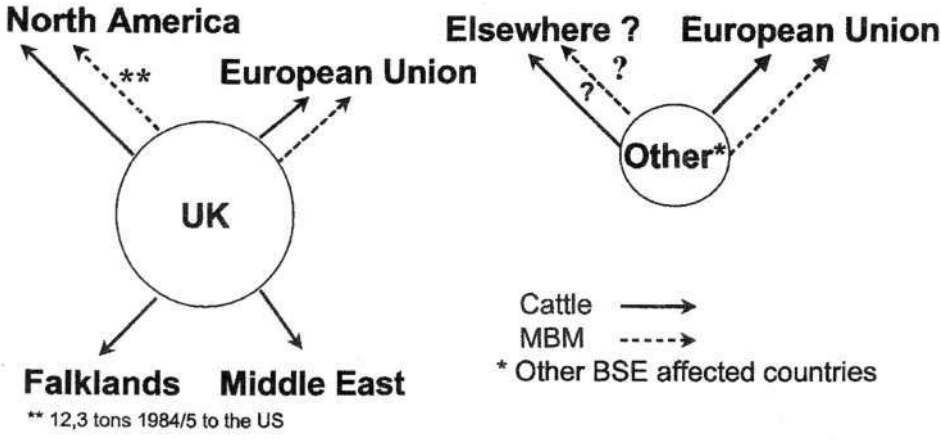

Fig. 4. Exports of cattle and meat-and-bone meal from BSE-affected countries in the 1980s

CATEGORISATION OF TSE RISK OF TISSUES BY THE WHO

At a Consultation in 1997, the WHO classified bovine body tissues and body fluids (on the basis of relative scrapie infectivity in sheep and goats with clinical disease) into one of four categories (I - IV) (22), with CATEGORY I having high infectivity, CATEGORY II medium infectivity, CATEGORY III low infectivity and CATEGORY IV no detectable infectivity. The Committee for Proprietary Medicinal Products (CPMP) of the EU had produced a similar table earlier. The consequent classification of starting materials used in vaccine manufacture is listed in Tables III a and III b. Most starting materials used in vaccine manufacture are in CATEGORY IV but pancreas, the source of pancreatic extract, is in CATEGORY III and spleen is in CATEGORY II so these deserve and receive special attention. So do gelatin derivatives and amino acids derived from gelatin, glycerol and fatty acids as these are produced from mixed species materials either from bone or by rendering and additional further processing. Before discussing the TSE risks in these commodities it is necessary first to determine the cattle tissues in which BSE infectivity has been detected and those tissues in which no detectable infectivity is found, either in natural or experimental BSE.

Ta b 1 e III a. Major categories of *bovine/ovine* starting materials

CPMP/WHO RISK CATEGORY	FULL NAME	TISSUE
IV	Beef heart bovine	Heart
IV	Bovine meat extract	Skeletal muscle
IV	Heamaglobin	Blood
IV	Haematin	Blood
IV	Donor calf serum	Blood (live cattle)
IV	Fetal calf serum	Blood (killed fetuses)
IV	*Sheep blood*	Blood (live sheep)
IV	Skimmed milk	Milk (live cattle)
IV	Casein/Casein peptone/Casamino acids/ Lactose/Lactalbumin hydrolysate/ Galactose/Hycase/Casein hydrolysate	Milk (live cattle)

Table IIIb. Major categories of *bovine/ovine* raw materials (Continued)

CPMP/WHO RISK CATEGORY	FULL NAME	TISSUE
IV	Choline chloride	Gall bladder bile
IV	*Cholesterol*	Wool (lanolin)
IV	Sodium deoxycholate	Gall bladder bile
III	Pancreatic extract	Pancreas
II	Spleen derivative	Spleen
NC*	Glycerol	Tallow derivatives**
NC*	Gelatin derivatives	Bones
NC*	Tween (buffer components include fatty acids)	Tallow derivatives**

*: Not categorized
**: Tallow derivatives drived from multiple species

TISSUE INFECTIVITY IN NATURAL BSE IN CATTLE

The only tissues in which infectivity has been detected in naturally occurring, clinically affected, confirmed cases of BSE following bioassay in mice, are the brain, cervical and terminal spinal cord and the retina (23, 24). The infectivity titre in the brain is 10^6 cattle i/c ID_{50}/g. The equivalent titre in mice is $10^{3.3}$i/c ID_{50}/g(SAC Hawkins and GAH Wells, personal communication). This indicates that the mice can underestimate the amount of infectivity by about 500 times and may therefore be less effective at identifying low levels of BSE infectivity. This difference in sensitivity is attributed to the species barrier effect.

Some 50 or so tissues show no detectable infectivity in natural BSE disease including: milk (25), blood and blood components, fetal calf blood, spleen, skeletal muscle, heart, bone marrow, pancreas or any male or female reproductive tissue (23, 24, 26).

In a separate study, pooled spleen, and separately lymph nodes, from five cases of natural BSE (the same source cattle used to assay infectivity in brain in mice and cattle - see above) has failed to transmit BSE to cattle after challenge by the i/c route (SAC Hawkins and GAH Wells, personal communication).

TISSUE INFECTIVITY IN EXPERIMENTAL BSE IN CATTLE

Wells et al, (27, 28, 29) have reported a study of the tissue infectivity in cattle experimentally, orally-challenged with BSE in which over forty tissues were collected at approximately 4 monthly intervals during the incubation period, for bioassay in susceptible mice. A proportion of them are being also bio assayed in cattle by the intracerebral (i/c) route that eliminates the species barrier.

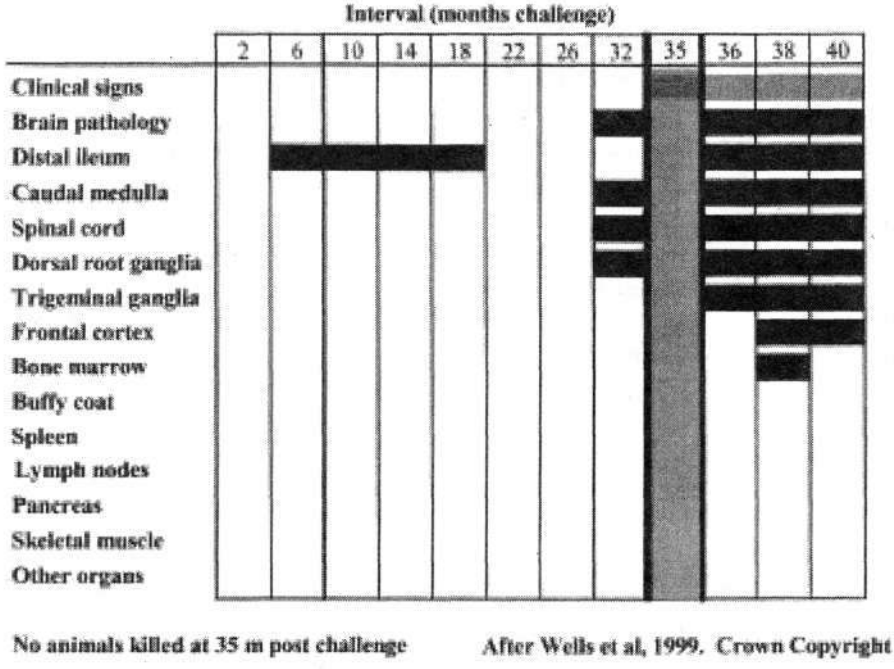

Fig. 5. Cattle experimentaly, orally-challenged with BSE:
Pathogenesis study in cattle, tissues bioassayed in mice

The mouse study is complete (FIGURE 5) and has revealed infectivity in central nervous tissues (from three months before clinical onset of disease in survivors) and in the distal ileum from six months after challenge (10 months of age). In the clinical stage of disease only, one sample of pooled sternal bone marrow showed detectable infectivity at 38 months post challenge, but not before or after this stage. Wells et al, (29) discussed the interpretation of this result in detail.

The bioassay in cattle of selected tissues and at selected stages of incubation is incomplete but to date no tissue negative in the mouse study has revealed infectivity in the cattle study. However, buffy coat from cattle challenged with BSE collected at 32 months post challenge has so far revealed no detectable infectivity after over four years from inoculation of cattle i/c (SAC Hawkins and GAH Wells, personal communication).

COMPARISON WITH NATURAL SCRAPIE IN SUFFOLK SHEEP

The tissue distribution of infectivity in natural scrapie in Suffolk sheep (30) shows a much wider distribution than in either natural or experimental BSE. In particular there is widespread infection of the lymphoreticular system detectable from about 10 months of age. The central nervous system is detectably infected from about 24 months of age, with clinical signs developing later still. Early in the BSE epidemic there were no transmission data so these scrapie data were used as the best estimate of what might be expected in cattle (22). However, the studies of Bruce et al (13) clearly showed that the agents that cause scrapie are different from the single major strain of agent that causes BSE. Furthermore the BSE agent appears stable and a consistent strain type causes BSE in different geographical regions of the UK, in Switzerland and presumably other countries where BSE has occurred. This is supported by the consistency of clinical signs, incubation period and lesion distribution within different cattle populations. Thus it now seems wise to rely upon data derived from cattle especially as the BSE agent differs from scrapie agents.

SPECIFIC STARTING MATERIALS

Milk

The WHO, OIE, EC, CPMP of the EU, the UK Spongiform Encephalopathy Advisory Committee have independently concluded from all the experimental and epidemiological evidence that milk is safe. In addition the US Department of Agriculture has placed no restriction on the importation of milk and milk products into the USA even from countries with BSE.

Blood

No detectable infectivity has been found in blood (including fetal calf blood) or any blood component in natural or experimental cases of BSE in cattle, or in any other blood-related tissue during the period of incubation (Table IV) (31).

Fetal calf serum or donor calf serum (from live animals) is used during the manufacture of human and veterinary vaccines. For some years since 1985, some 10 million doses of veterinary vaccines prepared using UK serum were distributed annually for use in cattle throughout Europe and not just in countries that have reported BSE. If BSE infectivity existed in this serum it would be more likely to be revealed in inoculated cattle because there would be no species barrier. However, epidemiological studies in the UK could not attribute the occurrence of BSE to the use of vaccines (19). Furthermore, cases of BSE in native-born cattle have occurred only in 12 other European countries and they are very few in number compared with the number of doses of vaccine distributed. It can therefore be concluded that the occurrence of BSE

Ta b 1 e IV. No detectable infectivity (NDI) in bovine blood

Tissue	Natural BSE		Experimental BSE	
	Tested in cattle	Tested in mice	Tested in cattle	Tested in mice
Blood clot		NDI		
Serum		NDI		
Buffy coat		NDI	NDI**	NDI
Fetal calf blood		NDI		
Spleen	NDI	NDI		NDI
Lymph mode	NDI	NDI		NDI
Bone marrow		NDI	(in progress)	NDI (during incubation)

* from 32 months incubating donor cattle, > 4 years after challenge of recipent cattle (experiment still in progress)

is not associated with the use of veterinary vaccines (and thus bovine serum from the UK) and that any TSE risk in them is negligible. Whatever is the negligible risk, it is likely to be smaller in human vaccines since a species barrier is imposed.

Skeletal muscle and pancreas

Skeletal muscle from three sites, heart muscle and pancreas from natural, confirmed cases of BSE have shown no detectable infectivity after bioassay in susceptible mice.

BSE infectivity has not been detected in skeletal muscle or pancreas at any stage of incubation of experimental BSE following bioassay in susceptible mice.

Tallow

Tallow, both filtered and unfiltered has shown no detectable TSE infectivity in experimental spiking studies using BSE-(32) and scrapie-infected brain material (33) from natural cases of disease. Furthermore, tallow as such is not used in the preparation of vaccines. Rather, tallow derivatives including fatty acids (for use in buffers) and glycerol are used. These are produced by processes, accepted by regulatory authorities, as sufficiently severe as to ensure safety in regard to TSE agents.

Gelatin derivatives and amino acids prepared from bovine bone

No inherent detectable infectivity has been detected in the bone marrow of clinically healthy cattle with experimental BSE or in clinically affected cattle with natural disease. However, the TSE risk in bones historically used for gelatin manufacture may not have been negligible due to contamination with TSE-infected CNS tissue. There are however, significant (c.10^7 times) reductions in titre as a result of the processing of gelatin from bones (Gelatin Manufacturers of Europe, personal communication). Furthermore, the gelatin used to prepare gelatin derivatives was not sourced from the UK. No incident of TSE of any kind has incriminated gelatin as the cause. No case of BSE has been attributed to gelatin despite being fed to cattle in the form of waste human food and used for coating feed additives.

Amino acids are prepared from gelatin by hydrolysis at 120°C using hydrochloric acid (HC1), pH 0.8 for 4 hours. Using an incubation time bioassay of hamster scrapie

brain ($10^{8.6}$ hamster i/c LD_{50}/g) treated with IN HCl for 1 hour at > 65°C leads to almost complete inactivation of infectivity (34).

Thus any residual TSE infectivity in gelatin is likely to be effectively inactivated by the subsequent acid treatment. It can therefore be concluded that amino acids prepared from gelatin carry a negligible TSE risk.

The risk analysis for all these starting materials concludes that any residual risks are negligible.

CONCLUSIONS

Because of the paramount importance of ensuring safe sources for all starting materials an exhaustive risk analysis is essential. Furthermore, the geographical approach alone is not sufficient due to the dynamic nature of risk development. A dynamic risk management policy is required to keep pace with changes in risk over time as a result of new information and as a result of the introduction of new legislation.

Real risks identified in any risk analysis must be effectively managed and communicated. Perceived risks must be managed too.

The qualitative assessment of risk in the circumstances described in this paper shows no evidence for a degree of risk that is higher than negligible.

The cumulative effect of all negligible risks does not increase the level of risk in any product to higher than a negligible level.

Cells banks and seeds do not contain any bovine material for which TSE infectivity has been demonstrated.

ADDITIONAL ACTIONS FOLLOWING THE RISK ANALYSIS

The 'global' programme of replacing materials of animal origin with alternative animal-free sources that have no additional risks will be continued.

The continued use of safe sources of essential animal starting materials with full traceability will be ensured.

Where the use of bovine materials is unavoidable, starting materials will be obtained only from live healthy animals (e.g. milk and donor blood) or from healthy animals killed under veterinary supervision, passed fit for human consumption and derived from countries that satisfy the conditions for freedom from BSE specified in the OIE, International animal Health Code chapter on BSE (7).

CONCLUDING REMARKS

The TSE safety of vaccines for human use, including those used to protect children from serious, and sometimes fatal, childhood illnesses and which are prepared using bovine tissues, can be assured by rigorous application of risk management procedures as determined in this risk analysis. Risk analysis, management and communication (including communication to regulatory bodies and the public) are dynamic processes that must be continuously re-assessed in the light of new information and experience. Any TSE risk in vaccines is currently assessed to be negligible provided the recommendations and risk management procedures are followed. The benefits of vaccines continue to outweigh, by a large margin, any risks that might derive from their use.

The rigour with which licensing authorities worldwide apply their interrogations is appreciated as this adds a further dimension to the need for scrupulous attention to detail. This also contributes to the maintenance and development of public confidence in vaccines.

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