Sixth Annual Report 1997

Creutzfeldt-Jakob Disease Surveillance in the UK

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Summary

The national surveillance programme for Creutzfeldt-Jakob disease (CJD) in the UK was initiated in May 1990. The information provided in this sixth report continues to provide evidence of a high level of case ascertainment and that detailed clinical and epidemiological information has been obtained for the great majority of patients. A high post mortem rate has been maintained through the period of the study 1990-1997. The success of the project continues to depend on the extraordinary level of co-operation from the neuroscience community and other medical and paramedical staff throughout the UK. We are particularly grateful to the relatives of patients for their help with this study.

The average number of cases of sporadic CJD identified annually since 1990 was higher than in previous surveillance periods extending back to 1970. It is impossible to say with certainty to what extent these changes reflect an improvement in case ascertainment and to what extent, if any, changes in incidence.

In England, Scotland and Wales mortality rates from sporadic CJD were, respectively, 0.7, 0.8 and 1.2/million/year. These rates are comparable to those observed in other countries in Europe and elsewhere in the world, including countries which are free of BSE. Mortality from sporadic CJD in Northern Ireland was lower (0.18/million/year). There was some variation in the rates between the different regions within Great Britain. The highest mortality from sporadic CJD was observed in the South-West region of England (SMR = 144). Previous analyses have found no convincing evidence of space-time clustering, and this remains the case for the analyses in this report.

A case-control analysis of occupational histories has revealed no evidence that any of the occupations considered on biological grounds to be potentially at higher risk of CJD were actually associated with an increased risk of CJD. Six cases of sporadic CJD occurring since May 1990 have been identified in farmers and their spouses, all of whom lived or worked on farms with cattle and who may have had contact with cattle affected by BSE. While this represents a higher incidence rate among cattle farmers than among the general population, the incidence rate in dairy farmers is not exceptional when compared with rates observed in dairy farmers in other European countries with little or no BSE. Transmission experiments in mice and analysis of prion protein subtypes in these cases indicate that the causal agent has transmission characteristics which are distinct from both new variant CJD (nvCJD) and BSE and that the protein deposited in the brain in these cases is also distinct from both nvCJD and BSE. The neuropathological profile is similar to that of cases of sporadic CJD.

In the analysis of dietary histories statistical associations were found between various meat/animal products and the risk of CJD. These apparent associations should be treated with great caution in view of the methodological problems of the case-control study including the difficulty of obtaining accurate dietary histories from relatives and the possibility that biases in the histories may be different for cases and controls. It is of note that apparent dietary risk factors have been found for sporadic CJD, a condition that is not thought to be causally linked to animal spongiform encephalopathies.

In this report information is included on the 23 cases of nvCJD that have been identified in the UK up to 1st January 1998. Analyses which take account of delays in reporting and confirmation have found no evidence that the incidence rate of nvCJD has increased over the period 1994-1997. Analysis of the places and times of occurrence of nvCJD cases does not provide evidence of space-time clustering. The case-control study based on data from 23 cases of nvCJD and 19 age- and sex-matched hospital controls does not provide evidence of an increased risk in relation to prior medical treatment, occupation or diet. The dietary data does however suggest that the UK population in the age range from mid teens to forties may have undergone widespread exposure to the BSE agent through dietary consumption of products containing mechanically recovered meat in the 1980s.

SECTION

2

2 Clinical Surveillance - Introduction

The national surveillance of CJD was initiated in May 1990 in response to a recommendation in the Report of the Working Party on Bovine Spongiform Encephalopathy (Southwood Committee). The surveillance is funded by the Department of Health and the Scottish Office. The primary aim of the project is to monitor cases of CJD in order to identify any change in the pattern of this disease that might be attributable to the emergence of bovine spongiform encephalopathy (BSE). This report documents the findings from the CJD Surveillance Project in relation to sporadic, familial and iatrogenic CJD up to 30th April 1997 and in relation to new variant CJD (nvCJD) up to 1st January 1998.

2.1 Sporadic Creutzfeldt-Jakob disease

Between 1st January 1970 and 30th April 1997, 679 cases of sporadic CJD were identified, of whom 5 cases were still alive on 30th April 1997. Of these, 546 (80%) were classified as definite cases with the remainder classed as probable. The number of deaths each year from sporadic CJD is shown for England and Wales since 1970 in Figure 1a and for Scotland and Northern Ireland since 1985 in Figure 1b. In England and Wales the number of deaths identified each year has increased from around 10 per year at the beginning of the 1970s to about 35 per year in the 1990s. No secular trend is apparent over the shorter time period for which data are available for Scotland and Northern Ireland. Over the period 1990-1996 annual mortality rates from sporadic CJD per million population were 0.68 in England, 1.19 in Wales, 0.76 in Scotland and 0.18 in Northern Ireland. There is some evidence of variation in recorded mortality between the different countries ($p = 0.03$). When Northern Ireland is excluded from the comparison the variation is no longer statistically significant ($p = 0.09$). The rates in England, Scotland and Wales are consistent with those observed in other European countries. It is not clear to what extent the low mortality rate observed in Northern Ireland represents low underlying mortality or lower case ascertainment there.

Figure 1a **Deaths from sporadic CJD, England and Wales, 1970-1996**

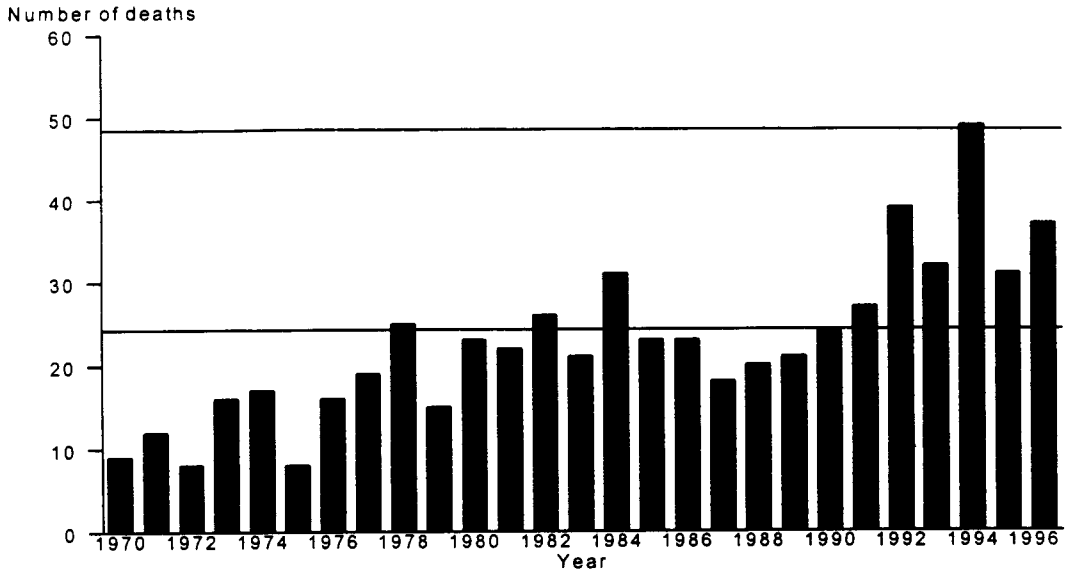
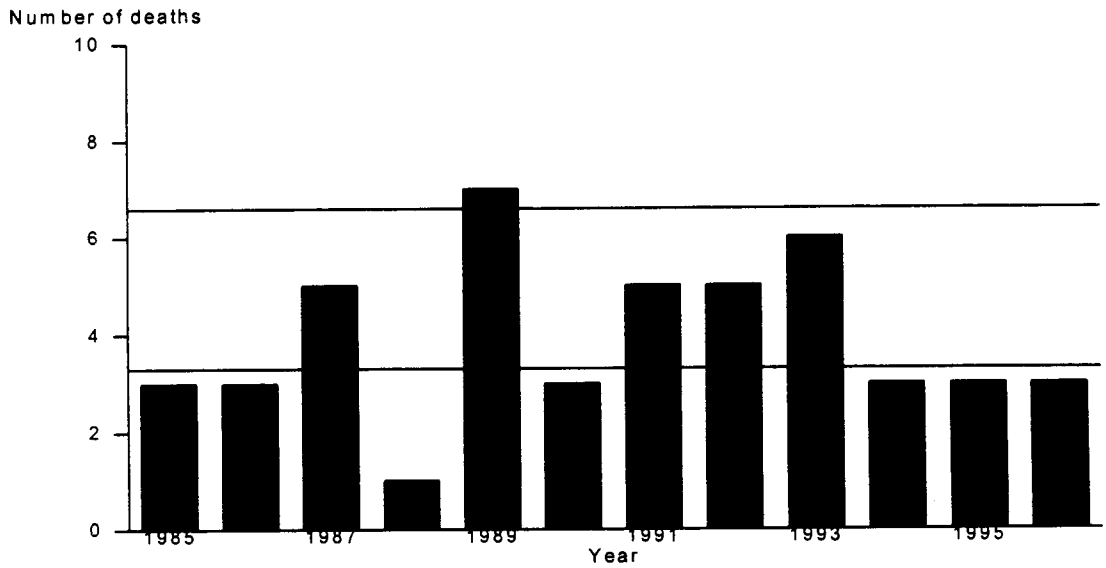


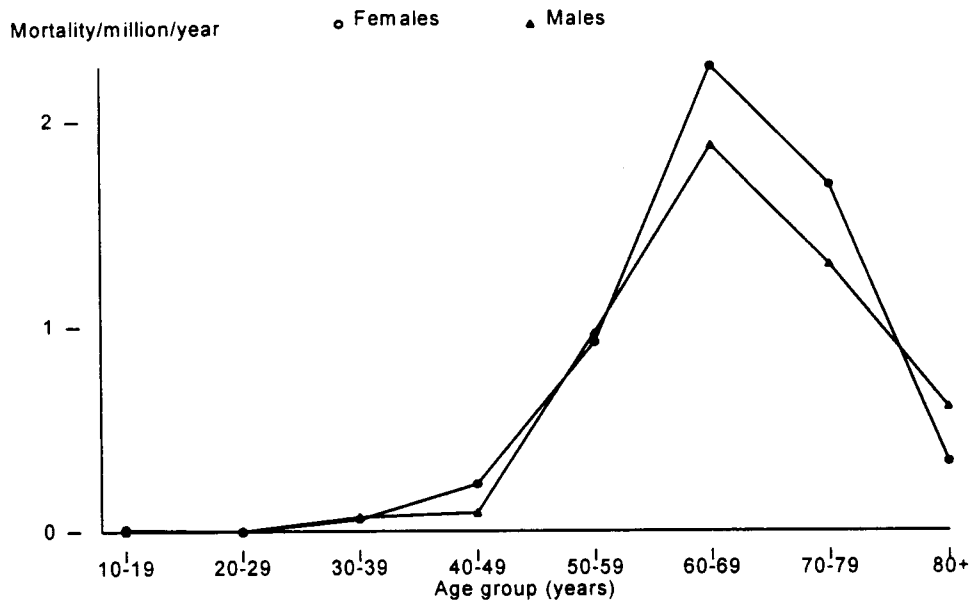
Figure 1b **Deaths from sporadic CJD, Scotland and Northern Ireland, 1985-1996**



Note: the horizontal lines indicate the number of deaths corresponding to mortality rates of ½ and 1 per million per year.

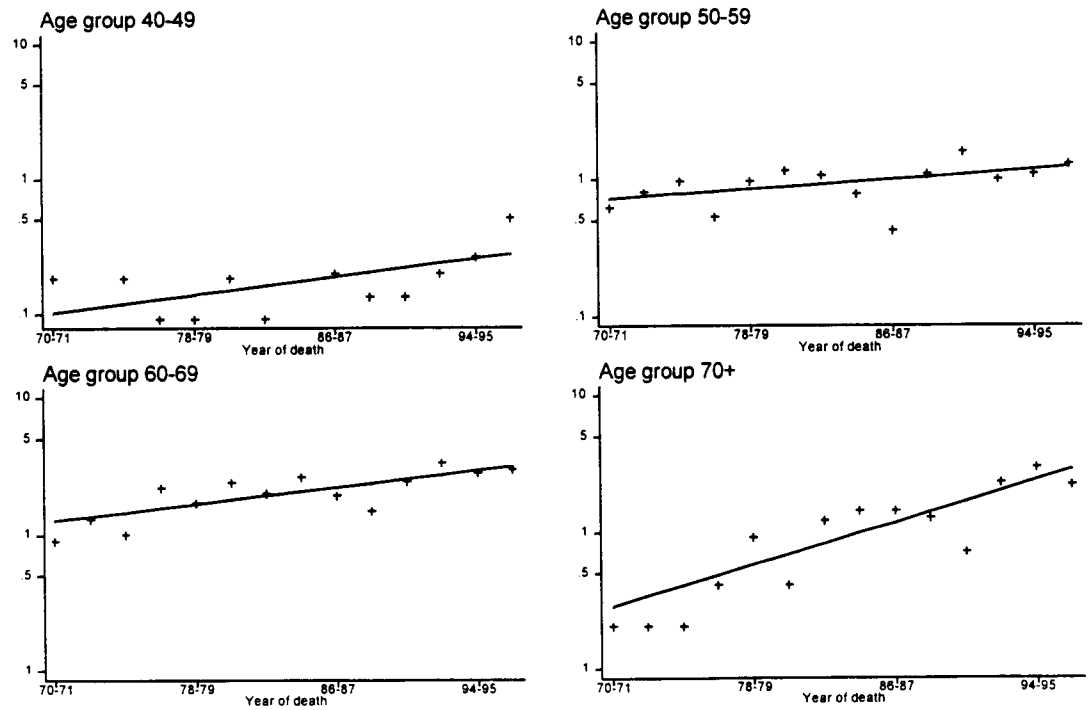
Figure 2 shows average annual age- and sex-specific mortality rates over the study period (1970-96). The median age at death was 64 years. Below 40 years of age mortality rates were extremely low (< 0.1/million/year). Thereafter they increased, reaching a peak of around 2/million/year in the age group 60-69, after which they declined. This decline in the oldest age groups has been noted in other countries and a possible explanation for this phenomenon is poorer case ascertainment in these older age groups, when other causes of dementia are much more common.

Figure 2 **Age- and sex-specific mortality rates from sporadic CJD in the UK, 1970-1996**



An analysis of age specific trends over the period since 1970 (Figure 3) shows that the greatest relative increase in mortality has occurred in those aged 70 years and above, and that currently the mortality rate in this age group is similar to that in the age group 60-69 years. This observation is consistent with improved case ascertainment in the elderly in recent years. There have been a slight increases in incidence in the age groups 40-49 and 50-59 but these are not statistically significant.

Figure 3 **Trends in mortality from sporadic CJD by age**



Note: Rates are shown per million per year on a logarithmic scale.

Table 1 presents the numbers of deaths underlying these trends. These data emphasise the very small numbers of cases of sporadic CJD occurring in individuals aged less than 50 years and show very clearly the substantial increase in the numbers of deaths identified among those aged 70 years and above, from around one per year in England and Wales in the early 1970s to around 15 per year in the UK currently.

Standardised mortality ratios (SMRs) for the standard regions of Great Britain for the period 1st January 1985 to 30th April 1997 are shown in Figure 4. There was some evidence that, after adjusting for the age/sex distribution of the population, mortality rates varied between the different regions (p=0.04). Highest mortality was observed in the South West region (SMR=144), Scotland (SMR=128) and Wales (SMR=124). Lowest mortality was observed in the West Midlands (SMR=62), the East Midlands (SMR=77) and the North (SMR=88). The SMRs for the other four regions all lay between 90 and 110. The highest SMR (=144 in the South West) arose from 51 cases observed compared with 35.3 expected (on the basis of rates for the whole of Great Britain), an excess of about 16 cases over a period of just over 12 years. In Scotland and Wales the excess numbers of cases over "expected" were approximately 10 and 5 respectively.

Figure 4 **Standardised mortality ratios (SMRs) by standard region, Great Britain, 1985 - April 1997**

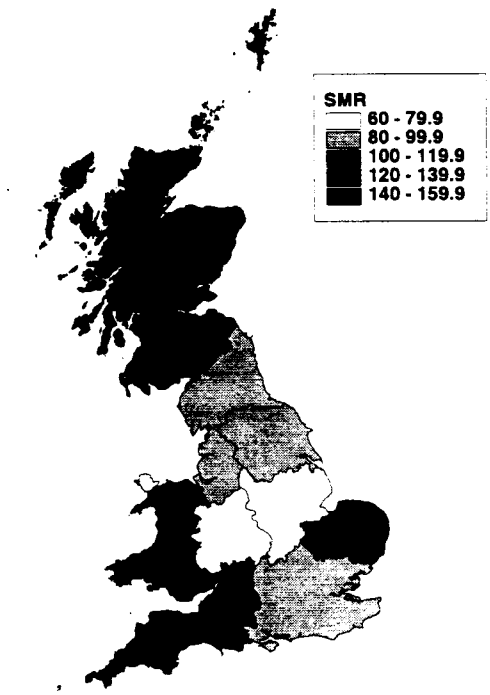


Table 1 Cases of sporadic Creutzfeldt-Jakob Disease in England and Wales (from 1970) and the U.K. (from 1985)

Age at death (years)	Year of death														**Total[2]**
	70-71	72-73	74-75	76-77	78-79	80-81	82-83	84-85[1]	86-87	88-89	90-91	92-93	94-95	96-97[2]	
10-19	0	0	0	0	0	1	0	0	0	0	0	0	0	0(0)	**1(0)**
20-29	0	0	0	0	0	0	0	0	0	0	0	0	0	0(0)	**0(0)**
30-39	1	0	0	2	2	1	1	4	1	0	1	0	0	0(0)	**13(0)**
40-49	2	0	2	1	1	2	1	0	3	2	2	3	4	5(2)	**28(2)**
50-59	7	9	11	6	11	13	12	9	5	13	19	12	13	10(3)	**150(3)**
60-69	9	13	10	22	17	24	20	28	22	17	28	38	32	22(0)	**302(0)**
70 +	2	2	2	4	9	4	13	16	18	16	9	29	37	18(0)	**179(0)**
Total	**21**	**24**	**25**	**35**	**40**	**45**	**47**	**57**	**49**	**49[3]**	**59**	**82**	**86**	**55(5)**	**674(5)**

[1] Up to 1984, cases from England and Wales only. From 1985 onwards, cases from Scotland and Northern Ireland are included

[2] Deaths up to 30th April 1997. Numbers in parentheses indicate additional cases alive on 30th April 1997

[3] Total (49) includes one case whose age at death was unknown.

2.2 New Variant Creutzfeldt-Jakob disease

As of 1st January 1998, 23 cases of nvCJD had been identified in the UK (22 definite, one probable), all of whom had died. Thirteen of these cases were women. The median age at onset of disease was 28 years and the median age at death 29 years (compared with 64 years for sporadic CJD). The youngest case was aged 16 years at onset while the oldest case was aged 48 years. The average delay between onset of disease and confirmation of the diagnosis of nvCJD was 15.8 months. This has not decreased over time. Currently cases of nvCJD appear to be occurring at a steady rate (Figure 5). Analyses which take account of delays in reporting and confirmation have found no evidence that the incidence rate of nvCJD has increased during the period 1994-1997 (P.Farrington, personal communication).

Figure 6 shows the geographical distribution by place of residence at onset of the 22 cases of nvCJD with onset in Great Britain. This shows that the cases to date have been widely spread geographically.

Figure 5 **Cases of nvCJD by date of onset**

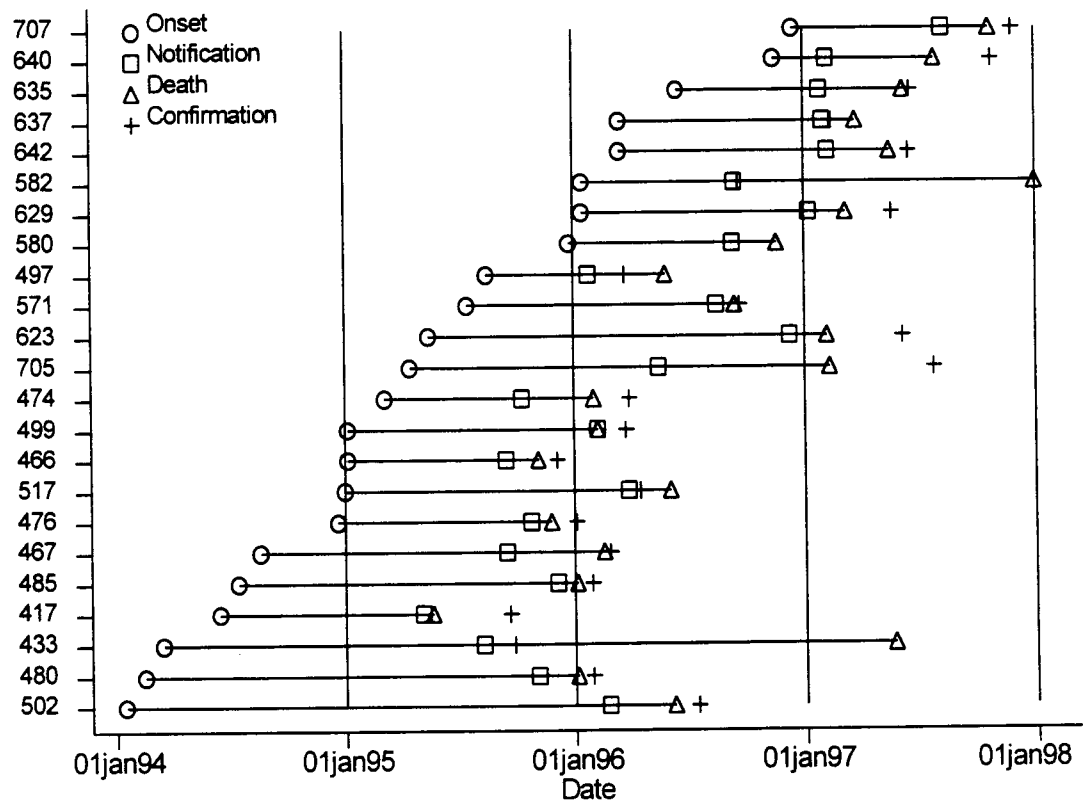


Figure 6 Geographical distribution by place of residence at onset of 22 cases of nvCJD

2.3 Iatrogenic Creutzfeldt-Jakob disease

Since 1970, up to 30th April 1997, 31 cases of CJD attributable to iatrogenic exposure have been identified, 6 in individuals receiving dura mater implants and 25 in individuals who had received human-derived growth hormone or gonadotrophin. The mean age at death for the hGH/hGNH cases was 28.7 years (with a range of 20-45 years) and for the dura mater cases was 43.3 (range 27-59 years).

The first identified iatrogenic case was a dura mater recipient who died in 1979. The first hGH-related death occurred in 1985. Between 1st May 1990 and 30th April 1997 there were 17 CJD deaths in hGH recipients, an average of about 2.5 deaths per year.

2.4 Familial Creutzfeldt-Jakob disease

The following figures do not include cases of GSS. Twenty-six cases of familial CJD and fatal familial insomnia (FFI) have been identified since 1970. Of these, 25 were resident in England and one was resident in Wales. Ten of the cases had insertions in the coding region of the PrP gene, 6 carried the mutation at codon 200 (Glu-Lys), 2 carried the mutation at codon 178 (Asp-Asn, both with methionine at codon 129) and 8 were identified as familial on the basis of relatives known to have had CJD (one with a relative known to have an insertion, one with a relative known to have the codon 200 mutation). The average age at death was 55 years.

2.5 An analysis of clustering of CJD in space and time

The method of Knox was used to look for evidence of clustering of cases of CJD in space and time (which might provide evidence of case-to-case transmission or a common source of infection, which was non-uniform geographically and temporally). Cases of sporadic CJD and of new variant CJD were analysed separately.

Sporadic CJD

Three hundred and ninety cases of sporadic CJD identified in Great Britain, with onset on or after 1st January 1985, were included in the analyses. When known, the date of clinical onset was used as the time point in the analyses. When date of onset was unknown (approximately 3% of cases), it was set at 4 months prior to the date of death (4 months being the median duration from onset to death for cases with known date of onset).

The results of the Knox analyses of these 390 cases are shown in Table 2. For most space-time combinations the observed number of pairs is close to the number expected under the null hypothesis (no space-time clustering). For only one combination is there a "significant" excess of the observed number of pairs over that expected. Eighty-nine pairs were observed to occur within 10km of each other with onset 3 to 4 years apart (compared with about 73 such pairs expected, p=0.04). Forty of these observed pairs occurred in three "clusters". Two of these clusters occurred in the Greater London area (one of 15 cases forming 23 pairs over the period 1986-1994, the other of 8 cases forming 9 pairs over the period 1985-1996). The third "cluster" occurred in the Glasgow area (6 cases forming 8 pairs over the period 1987-1994).

Table 2 Space-time clustering of dates and places of onset of 390 cases of sporadic Creutzfeldt-Jakob disease in Great Britain, with onset between January 1985 and April 1997: observed and expected numbers of pairs of cases with onsets within "critical" time and space distances of each other

Time[1] between dates of onset	Distance between places of residence at onset							
	< 5km		< 10 km		< 20 km		< 50km	
	Obs	Exp	Obs	Exp	Obs	Exp	Obs	Exp
< 1 month	5	6.6	10	11.5	29	30.2	86	88.3
1-3 months	11	9.8	20	17.2	41	45.4	118	132.6
3-6 months	16	15.0	31	26.2	78	69.1	213	201.7
6-12 months	24	29.2	51	51.1	136	134.9	372	394.1
1-2 years	52	54.3	95	95.0	235	250.9	682	732.7
2-3 years	48	48.5	73	84.7	231	223.7	677	653.4
3-4 years	51	41.6	89*	72.6	193	191.8	552	560.3
4-5 years	39	34.9	69	61.0	169	161.0	441	470.1

[1] Critical times used were (in days): 35, 95, 185, 370, 735, 1100, 1465, 1830

* $0.01 < p \leq 0.05$

An analysis of 622 cases of sporadic CJD occurring in England and Wales over the past 27 years (since 1970) is presented in Table 3. (During the period 1970 to 1984 only cases in England and Wales were recorded.)

Table 3 Space-time clustering of dates and places of onset of 622 cases of sporadic Creutzfeldt-Jakob disease in England and Wales, January 1970 to April 1997: observed and expected numbers of pairs of cases with onsets within "critical" time and space distances of each other

Time[1] between dates of onset	Distance between places of residence at onset							
	< 5 km		< 10 km		< 20 km		< 50 km	
	Obs	Exp	Obs	Exp	Obs	Exp	Obs	Exp
< 1 month	4	6.9	10	16.0	43	45.2	128	141.6
1-3 months	11	10.1	23	23.6	56	66.7	182	209.0
3-6 months	20	15.6	39	36.4	109	102.7	344	321.9
6-12 months	33	30.7	75	71.9	224	202.7	626	635.3
1-2 years	62	59.2	132	138.4	386	390.6	1168	1223.8
2-3 years	56	55.6	115	130.0	375	366.9	1196	1149.8
3-4 years	65*	50.4	122	117.9	334	332.6	1054	1042.1
4-5 years	56	46.2	111	108.0	300	304.6	959	954.5
5-6 years	50	43.1	105	100.8	306	284.4	921	891.3
6-7 years	31	34.3	81	81.3	235	235.1	745	723.5
7-8 years	32	41.0	89	95.8	271	270.3	885	847.0
8-9 years	49	39.7	102	92.7	282	261.5	856	819.5
9-10 years	29	39.5	83	92.3	266	260.5	845	816.2
10-15 years	120	154.5	335	361.2	959	1019.2	3116	3193.6
15-20 years	81	89.6	244**	209.4	628	590.7	1897	1851.0

[1] Critical times used were (in days): 35, 95, 185, 370, 735, 1100, 1465, 1830, 2195, 2560, 2925, 3285, 3655, 5490, 7310

* $0.01 < p \leq 0.05$

** $p \leq 0.01$

There is no convincing evidence of space-time clustering of cases in these data. Only two cells had an excess of observed pairs statistically significant at the 5% level. Sixty-five pairs of cases with onset 3-4 years apart , living within 5 km of each other, were observed compared with about the 50 such pairs expected (p=0.03). The largest "cluster" of cases responsible for these pairs was 8 cases in the London area who formed 15 pairs (a subset of the "cluster" of 15 cases mentioned above). Two hundred and forty-four pairs of cases with onsets 15-20 years apart, living within 10 km of each other, were identified, compared with an expected 209.4 such pairs (p=0.01). This excess appeared to result largely from cases which occurred in London during the 1970s being paired with cases occurring in London 15-20 years later.

Given the large numbers of cells in the two tables above (Tables 2 & 3), it is not surprising that a few cells contain apparent excess pairs of cases which are "statistically significant" at the 5% level. That these excesses tend to include "clusters" of cases in Greater London may be artefactual, arising from the use of a central grid reference for some cases (when the precise place of residence was not recorded).

New variant CJD

Similar analyses were performed for the 22 cases of new variant CJD identified in Great Britain up to 31st December 1997. The results of these analyses are presented in Table 4. None of the cells produced an excess of observed over expected cases statistically significant at the 5% level.

Table 4 Space-time clustering of dates and places of onset of 22 cases of new variant Creutzfeldt-Jakob disease in Great Britain, identified up to 31st December 1997: observed and expected numbers of pairs of cases with onsets within "critical" time and space distances of each other

Time[1] between dates of onset	Distance between places of residence at onset							
	< 5km		< 10 km		< 20 km		< 50km	
	Obs	Exp	Obs	Exp	Obs	Exp	Obs	Exp
< 1 month	0	0.1	0	0.1	0	0.2	1	0.6
1-3 months	0	0.1	0	0.1	0	0.3	2	1.0
3-6 months	0	0.1	0	0.1	0	0.4	2	1.5
6-12 months	0	0.3	0	0.3	0	0.8	0	3.1
1-2 years	1	0.4	1	0.4	3	1.1	6	3.9

[1] Critical times used were (in days): 35, 95, 185, 370, 735

SECTION

3

Case-Control Study

Methods

A case-control study of CJD has been carried out in the UK since May 1990 to investigate individual risk factors for the disease. Relatives of patients with suspect CJD are interviewed using a standard questionnaire which includes a wide range of questions relating to putative risk factors for CJD including occupational history and dietary history. For each suspect case, a patient at the same hospital, matched for sex and age $\pm$ 4 years, is identified as a control. Individuals with diseases which might be confused clinically with CJD are excluded from being controls. When possible, a relative of the same degree as for the case is interviewed using the standard questionnaire. If this is not possible the control is interviewed directly. Since May 1990 a relative of the control has been interviewed on 54% of occasions and the control themselves has been interviewed on 46% of occasions.

Dietary history and the risk of CJD: some caveats

The findings of the case-control study concerning dietary history and risk of CJD are particularly difficult to interpret for a number of reasons.

1. It is known that accurate dietary histories are very difficult to obtain, even directly from individuals about their own diet, particularly when information is required on eating habits over many years previously. These problems are exacerbated when information must be obtained from a relative rather than directly from the individual themselves. Thus there is substantial potential for obtaining incorrect information (misclassification) in the dietary component of the study.

2. At the time of interview, the relatives are aware that a diagnosis of CJD is suspected and they are often aware of the dietary hypotheses being investigated (because of the widespread reporting in the media of the possible link between CJD and BSE even prior to 1996). This may influence their responses to questions about diet. Thus, there is substantial potential for obtaining biased responses with respect to diet (recall bias).

3. The small number of cases in the case-control study and the relative homogeneity of reported consumption of certain food items, have resulted in imprecise estimates of associations and variable findings from one year to the next. For example, in the Second Annual Report (1993) pudding (black pudding) consumption appeared to be a dietary risk factor for Creutzfeldt-Jakob disease but in subsequent years no evidence of such an association has been observed.

Results

Sporadic CJD

206 cases of classical, sporadic Creutzfeldt-Jakob disease identified between May 1990 and April 1997 (75% of all such cases ascertained during this period) were compared with age-, sex- and hospital-matched controls with respect to lifetime history of eating of a wide range of animal tissues and products. Consumption was recorded as follows:

- never,
- less than once per year,
- more than once per year but less than monthly,
- more than once per month but less than once per week,
- once per week or more,
- eaten but frequency unknown.

For all 206 cases and for 111 (54%) controls, information on dietary history was obtained from a relative. For the remaining 95 (46%) controls, information was obtained from the control themselves. In addition to collecting information on lifetime dietary history, dietary history since 1985 was also recorded for 194 (94%) of the case-control pairs for some specific animal tissues/products. The analyses were performed using the computer package STATA and all estimates take account of the individual matching.

Diet

The results of the comparison of 206 case-control pairs with regard to lifetime consumption of different types of meat is shown in Table 5. Evidence of "dose-response" relationships was sought by fitting the consumption categories described above as a continuous variable (scoring the different categories as 0, 1, 2, 3 - excluding the "unknown frequency" category) and is indicated by the trend test shown in the table. For lamb, pork, beef, poultry and fish the category "never eaten" was excluded from these analyses of trend (ie the test for trend is among those who had ever eaten the item). For veal and venison the category "never eaten" was included in the analysis of trend, since its exclusion would have resulted in the loss of a large number of case-control pairs from the analysis. Thus, only one p-value (for the test for trend) is presented for these items.

Pork, poultry and fish were consumed by all or almost all cases and controls. There was no evidence that cases consumed these items more often than controls. Lamb and beef were also reported to be consumed by most cases and controls. More controls than cases were reported to have never eaten lamb ($p=0.06$). Among individuals reported to have eaten lamb, however, there was no evidence that cases ate it more often than controls. Only one control was reported as never having eaten beef. Cases were reported to consume beef more often than controls (trend: $p=0.02$). Consumption of venison and veal was much less common among both cases and controls than was

the consumption of other meats. Cases were reported to eat both of these meats more often than controls (test for trend; venison, p = 0.006; veal, p = 0.005).

Table 5 Results of a comparison between 206 cases of classic, sporadic Creutzfeldt-Jakob disease, post April 1990, and their matched controls with regard to lifetime history of eating different types of meat

Type of meat	Cons-umed	Number of cases (%)	Number of controls (%)	Odds ratio	95% c.i.	p-value (OR=1)	p-value[1] (trend)
Lamb	No	2 (1)	8 (4)	1.00	-	0.06	0.23
	Yes	202 (99)	195 (96)	4.00	(0.80,38.7)		
Pork	No	0 (0)	2 (1)	1.00	-	0.16	0.48
	Yes	203(100)	201 (99)	∞	(0.19,∞)		
Beef	No	0 (0)	1 (0.5)	1.00	-	0.32	0.02
	Yes	203(100)	202 (99.5)	∞	(0.03,∞)		
Venison	No	136 (68)	160 (78)	1.00	-		0.006[2]
	Yes	65 (32)	46 (22)	1.80	(1.08,3.06)		
Veal	No	120 (60)	143 (71)	1.00	-		0.005[2]
	Yes	80 (40)	59 (29)	1.69	(1.05,2.77)		
Poultry	No	0 (0)	0 (0)	-	-	-	0.88
	Yes	201(100)	203(100)	-	-		
Fish	No	2 (1)	0 (0)	1.00	-	0.16	0.28
	Yes	200 (99)	201(100)	0.00	(0.00,5.32)		

In order to investigate further the associations observed (for lamb, beef, venison and veal), the consumption data were regrouped into the following categories:

- lamb and beef; less than monthly, at least monthly, weekly;
- venison and veal; never, less than yearly, yearly.

(Regrouping was performed because with the original five categories used the data were sparse in some categories).

[1] For lamb, pork, beef, poultry and fish the test for trend excludes the category "never eaten". For venison and veal the category "never eaten" is included in the analysis.

[2] See comment in text.

Table 6 presents the results of an analysis of these data. As before, there is statistical evidence suggesting that the risk of CJD increases with the reported frequency of consumption of beef (p=0.01), venison (p=0.004) and veal (p=0.01). For beef there is an approximately 2½-fold increase in risk associated with eating beef every week compared with eating it less than once per month. The largest odds ratio is for venison, with individuals who eat venison at least once per year apparently having an 8-fold increased risk of sporadic CJD compared with individuals who never eat venison. It should be noted, however, that the 95% confidence interval around this estimate is very wide. Although individuals consuming lamb on a monthly basis appear to be at increased risk compared with individuals eating lamb less often than that (odds ratio =1.93; 95% c.i. 1.18, 3.17), the risk appears to decrease in those eating lamb on a weekly basis and overall there is no convincing evidence for an association between reported consumption of lamb and risk of sporadic CJD (test for trend: p=0.19). When the analysis is restricted to only those case-control pairs in which a relative of the control was interviewed, the overall pattern of the results remains largely unchanged (Table 7).

In addition to data on frequency of eating different types of meat, data were also collected on the frequency of eating other animal tissues or products. Table 8 presents a comparison of cases and controls with regard to lifetime consumption of other products investigated. (Only one case and one control were reported to have ever eaten eyes and these data are not shown in the table.)

Table 6 Results of an analysis of trends in consumption of lamb, beef, venison and veal between cases of classic, sporadic Creutzfeldt-Jakob disease, post April 1990, and their matched controls

Type of meat	Frequency of consumption	Number of cases (%)	Number of controls (%)	Odds ratio	95% c.i.	p-value (trend)
Lamb	< monthly	44 (22)	67 (33)	1.00	-	
	monthly	117 (58)	93 (46)	1.93	(1.18,3.17)	0.19
	weekly	41 (20)	42 (21)	1.33	(0.77,2.32)	
Beef	< monthly	17 (8)	34 (17)	1.00	-	
	monthly	84 (42)	88 (43)	1.80	(0.92,3.54)	0.01
	weekly	100 (50)	81 (40)	2.37	(1.21,4.68)	
Venison	never	136 (68)	160 (78)	1.00	-	
	< yearly	47 (23)	42 (20)	1.35	(0.79,2.29)	0.004
	yearly	17 (9)	4 (2)	7.94	(1.81,34.9)	
Veal	never	120 (60)	143 (71)	1.00	-	
	< yearly	51 (25)	44 (22)	1.38	(0.81,2.34)	0.01
	yearly	29 (15)	15 (7)	2.57	(1.22,5.44)	

Table 7 Results of an analysis of trends in consumption of lamb, beef, venison and veal comparing cases of classic, sporadic Creutzfeldt-Jakob disease, post April 1990, and their matched controls, for those 111 pairs with data obtained from relatives.

Type of meat	Frequency of consumption	Odds ratio	95% confidence interval
Lamb	< monthly	1.00	
	monthly	1.44	(0.72,2.85)
	weekly	1.20	(0.56,2.55)
Beef	< monthly	1.00	
	monthly	2.55	(0.88,7.47)
	weekly	3.72	(1.31,10.6)
Venison	never	1.00	
	< yearly	2.00	(0.94,4.27)
	yearly	∞	(0.18, ∞)
Veal	never	1.00	
	< yearly	1.74	(0.78,3.89)
	yearly	2.03	(0.80,5.16)

Almost all cases and controls were reported to have eaten sausages at some time and there was no evidence of any dose-response relationship. Similar proportions of cases and controls were reported to have eaten tripe, liver, trotters, puddings and heart (odds ratios all 1.30 or less), with no evidence of a dose-response relationship for any of these items. More cases than controls were reported to have eaten kidney, sweetbreads, tongue and haggis (odds ratios in the range 1.40-1.75), but these differences were not statistically significant. Nor was there evidence of any dose-response relationships for any of these items. More cases than controls were reported to have eaten brain (odds ratio of 3.13, $p = 0.005$).

Table 8 Results of a comparison between 206 cases of classic sporadic Creutzfeldt-Jakob disease, post April 1990, and their matched controls with regard to lifetime history of eating various animal products

Type of product	Cons-umed	Number of cases (%)	Number of controls (%)	Odds ratio	95% c.i.	p-value (OR=1)	p-value[3] (trend)
Sausage	No	3 (2)	6 (3)	1.00	-	0.48	0.48
	Yes	196 (98)	197 (97)	1.67	(0.32,10.7)		
Tripe	No	131 (65)	134 (67)	1.00	-	0.48	(0.421)
	Yes	70 (34)	65 (33)	1.18	(0.74,1.88)		
Liver	No	20 (10)	24 (12)	1.00	-	0.51	(0.77)
	Yes	182 (90)	178 (88)	1.25	(0.65,2.41)		
Kidney	No	60 (30)	76 (37)	1.00	-	0.08	(0.53)
	Yes	141 (70)	127 (63)	1.50	(0.96,2.35)		
Sweet-breads	No	169 (86)	182 (91)	1.00	-	0.11	(0.07)
	Yes	27 (14)	19 (9)	1.71	(0.89,3.31)		
Tongue	No	74 (38)	87 (43)	1.00	-	0.18	(0.92)
	Yes	122 (62)	116 (57)	1.41	(0.86,2.30)		
Brain	No	167 (86)	193 (94)	1.00	-	0.005	(0.02)
	Yes	28 (14)	12 (6)	3.13	(1.41,6.93)		
Trotters	No	143 (73)	147 (73)	1.00	-	0.81	(0.31)
	Yes	53 (27)	54 (27)	1.06	(0.66,1.71)		
Puddings	No	78 (39)	83 (41)	1.00	-	0.81	(0.26)
	Yes	120 (61)	119 (59)	1.06	(0.66,1.71)		
Haggis	No	125 (63)	144 (70)	1.00	-	0.09	(0.21)
	Yes	74 (37)	61 (30)	1.50	(0.95,2.38)		
Heart	No	124 (63)	134 (68)	1.00	-	0.25	(0.23)
	Yes	74 (37)	64 (32)	1.30	(0.83,2.05)		

Table 9 presents a more detailed analysis of the association between lifetime consumption of brains and risk of CJD, with consumption regrouped into 3 categories: never; less than once per year; once per year or more.

[3] For sausage the test for trend excludes the category "never eaten". For all other items the category "never eaten" is included in the analysis of trend, and the two p-values are not, therefore, independent of each other.

Table 9 Results of analysis of trends in lifetime consumption of brains between cases of classic sporadic Creutzfeldt-Jakob disease, post April 1990, and their matched controls

Type of meat	Frequency of consumption	Number of cases (%)	Number of controls (%)	Odds ratio	95% c.i.	p-value (trend)
Brains	never	167 (86)	193 (95)	1.00	-	0.009
	< yearly	20 (10)	10 (5)	2.75	(1.13,6.66)	
	yearly	7 (4)	2 (1)	4.02	(0.81,19.8)	

When categorised in this way, compared with people reported to have never eaten brain, individuals with occasional exposure (< 1/year) were estimated to have a 2¾-fold increased risk while those who had eaten brain once a year or more often had a 4-fold increase in risk (test for trend: $p = 0.009$). Restricting attention to the subset of case-control pairs with exposure data obtained from relatives produced broadly similar results.

Data on consumption, post-1985, of the animal tissues and products included in Table 8 were available for 194 case-control pairs. (Data on meat consumption post-1985 were not collected for some case-control pairs.) There was no statistical evidence of an association between consumption since 1985 of any of the animal products listed in Table 8 and risk of CJD ($p \geq 0.10$ for all products). Four cases and 3 controls were reported to have eaten brains during this period (odds ratio = 1.33 [0.23,9.10], p=0.7).

It is likely that many of the dietary exposures considered are associated in the population. For example, individuals who eat liver may be more likely also to eat kidney than individuals who do not eat liver. Thus there is potential for confounding of associations between particular dietary items and risk of CJD. In order to overcome this problem we modelled simultaneously the association between the different exposures identified above and risk of CJD. A model including 5 different items (lamb, beef, venison, veal, brains) was fitted. Each of these items was included as a variable with 3 levels of frequency (see Tables 6 and 9 above). In this model only two items showed evidence of independent dose-response relationships with risk of CJD; beef ($p = 0.05$) and brain ($p = 0.04$) (Table 10).

Table 10 Results of a simultaneous analysis of trends in lifetime consumption of beef and brains between 187 cases of classic sporadic Creutzfeldt-Jakob disease, post April 1990, and their age- and sex-matched controls

Type of meat	Frequency of consumption	Odds ratio[4]	95% c.i.	p-value (trend)
Beef	< monthly	1.00	-	0.05
	monthly	1.79	(0.82,3.91)	
	weekly	2.23	(1.00,4.97)	
Brain	never	1.00	-	0.04
	< yearly	2.43	(0.83,7.18)	
	yearly	2.66	(0.47,15.1)	

In order to investigate the possibility that recall bias was responsible for the associations reported above, 206 sporadic CJD cases and 80 individuals in whom the suspected diagnosis of CJD was not subsequently confirmed were compared with regard to their reported consumption of beef and brains (Table 11). This comparison revealed no evidence of a dose-response relationship between either beef or brain consumption and risk of CJD. These data are consistent with the hypothesis that the associations observed when cases were compared with their matched, "non-suspect" controls arose through recall bias rather than a direct link between beef or brain consumption and risk of sporadic CJD. However, while these latter findings are consistent with there being no association, we cannot exclude the possibility that such associations exist.

In summary, the dietary case-control study of sporadic CJD has identified a number of statistical associations between the reported lifetime history of consumption of a number of food products, from a number of different animal species, and the risk of sporadic CJD. The strongest of these associations, statistically, are with beef and brain consumption. These apparent associations should, however, be interpreted with great caution in view of the methodological problems associated with this aspect of the case-control study. Data from individuals initially suspected of having CJD but subsequently accorded another diagnosis provide circumstantial evidence that these associations may reflect recall bias rather than a real, underlying link.

[4] Estimates of the odds ratio adjusted for lamb, venison and veal consumption.

Table 11 Results of analysis of trends in lifetime consumption of beef and brains between 206 cases of classic sporadic Creutzfeldt-Jakob disease, post April 1990, and 80 "non-cases"

Type of meat	Frequency of consumption	Number of cases (%)	Number of "non-cases" (%)	Odds ratio[4]	95% c.i.	p-value (trend)
Beef	< monthly	17 (8)	6 (8)	1.0		
	monthly	84 (42)	30 (40)	0.99	(0.36,2.74)	0.76
	weekly	100 (50)	39 (52)	0.90	(0.33,2.46)	
Brain	never	167 (86)	68 (90)	1.0		
	< yearly	20 (10)	5 (7)	1.63	(0.59,4.52)	0.37
	yearly	7 (4)	2 (3)	1.43	(0.29,7.03)	

[4] Based on an "unmatched" analysis.

Occupational history

The occupational histories of cases, controls, their spouses and their parents have been analysed to identify employment in the following areas: medical/nursing/dentistry and related professions, laboratory work involving animals; work in pharmaceutical laboratories; work in other research laboratories; livestock farming/veterinary medicine; work in abattoirs/butchers' shops or other direct contact with animal carcasses; other occupations involving contact with animal products (eg leatherworkers).

There was no evidence that cases were more likely than controls to have ever worked in any of the above categories (Table 12). Twenty-two cases compared with 23 controls had worked in the medical professions; 14 cases compared with 18 controls had worked on farms or in veterinary medicine; 7 cases compared with 9 controls had worked in abattoirs/butchers' shops. One case had worked in an animal laboratory and one case had worked in a pharmaceutical laboratory (all pre-1985). No other cases nor controls had ever worked in animal, pharmaceutical or other research laboratories. Slightly more cases than controls had worked in other occupations involving contact with animal products (21 versus 14; odds ratio = 1.64; p = 0.19).

Most cases, 197 (96%), and controls, 199 (97%), had been married at some point in their lives. There was no evidence that cases were more likely than controls to have had spouses who worked in the medical professions (10 cases, 15 controls) or farming (7 cases and 9 controls). No cases or controls had spouses who had worked in animal laboratories. One case and one control had spouses who had worked in a pharmaceutical laboratory while one case and one control had spouses who had worked in research laboratories. Eight cases compared with 3 controls had spouses who had worked in other occupations involving contact with animal products (odds ratio = 2.7, p = 0.13) while 9 cases compared with 2 controls had spouses who had

worked in abattoirs/butchers' shops (odds ratio = 8.0, p = 0.02). This latter finding is strange. It is not clear why being married to an abattoir worker or butcher should increase one's risk of sporadic CJD when working oneself in an abattoir or as a butcher is not associated with any increase in risk. This may be a chance finding.

Similar patterns were observed with regard to the occupations of parents of cases and controls. Excluding cases and controls who themselves or whose spouse worked in the following professions, only one case had a parent who had worked in a laboratory (research), 3 cases and 3 controls had parents who had worked in the medical professions, 7 cases and 12 controls had parents who had worked in farming/veterinary medicine, 5 cases compared with 4 controls had parents who had worked in abattoirs/butchers' shops while 6 cases and one control had parents who had worked in other occupations involving contact with animal products.

Table 12 Results of a comparison of 206 cases of classic sporadic CJD with their matched controls with regard to their lifetime occupational history and that of their spouse

Occupation	Exposure	Number(%) of cases	Number (%) of controls
Medical/paramedical /nursing/dentistry	Subject	22 (11)	23 (11)
	Spouse	10 (5)	15 (7)
Animal laboratory	Subject	1 (0.5)	0 (0)
	Spouse	0 (0)	0 (0)
Pharmaceutical laboratory	Subject	1 (0.5)	0 (0)
	Spouse	1 (0.5)	1 (0.5)
Research laboratory	Subject	0 (0)	0 (0)
	Spouse	1 (0.5)	1 (0.5)
Farmer/vet	Subject	14 (7)	18 (9)
	Spouse	7 (3)	9 (4)
Butcher/abattoir worker/other occupation with direct contact with animals/ carcasses	Subject	7 (3)	9 (4)
	Spouse	9 (4)	2 (1)
Occupation involving animal products	Subject	21 (10)	14 (7)
	Spouse	8 (4)	3 (1)

Analysis of specific occupations at the time of disease onset in sporadic CJD

Six cases of sporadic CJD occurring since May 1990 have been identified in farmers and their spouses, all of whom lived or worked on farms with cattle. Four of these occurred in individuals living or working on farms which had experienced a case of BSE and 4 cases were in dairy farmers. While this represents a higher incidence rate

among cattle farmers than among the general population, the incidence rate in dairy farmers (4/million/year) is not exceptional when compared with the rates observed in dairy farmers in other European countries with little or no BSE. Transmission experiments to mice using material from two of these six individuals have shown that the "strain" of the agent does not resemble the BSE agent in terms of its incubation period in mice or the profile of neuropathological lesions induced in the mice. Furthermore, Western blotting of PrP from 5 of the 6 cases indicates a different glycoform ratio in all 5 cases to that observed for new variant CJD and BSE. A more detailed analysis of these 6 cases has already been published (see Appendix 1).

New variant CJD

These analyses are based on data from 23 cases of new variant CJD and 19 age- and sex-matched hospital controls.

Medical history

Two cases had a definite history of blood transfusion. One had received blood following a road traffic accident some 18 years before disease onset, while the second had received an exchange transfusion at birth, some 24 years before disease onset. A further case might have received a transfusion during a hysterectomy about one year prior to disease onset. Two of the controls had also received blood transfusions in the past. Ten of the cases had no history of any operation/surgical procedure (other than dental procedures) compared with 4 of the controls. A list of surgical procedures undergone by cases and controls is presented in Table 13.

There is no evidence to suggest that cases were more likely than controls to have had operations in the past. This finding should be interpreted with some caution. Although operations directly related to the current admission were excluded from the analysis, the use of hospital controls for the study of potential iatrogenic risk factors is not optimal. This shortcoming will be addressed in future by the recruitment of community controls recruited from the same general practice as the case.

Table 13 List of operations undergone by 23 cases of new variant CJD and 19 hospital controls

Cases	Controls
Tonsillectomy (x 4)	Tonsillectomy (x 2)
Caesarian section (x 3)	Appendectomy (x 3)
Orthopaedic (x 2)	Caesarian section
Hysterectomy	Orthopaedic
Ectopic pregnancy	Sterilisation
Hernia	Vasectomy
Mastoidectomy	Gall bladder operation
Hydrocele	Removal of adenoids
Sutures to wound (x 5)	Removal of lump/nodule (x 2)
D + C	Sutures to wound (x 5)
Colonoscopy	Laparoscopy
Laparoscopy (x2)	Squint correction (x 2)
Gastric lavage	

Diet

The reported consumption of various different meats by cases and controls is shown in Table 14.

Table 14 Results of a comparison between 23 cases of new variant Creutzfeldt-Jakob disease and 19 controls with regard to consumption of different types of meat

	Ever		Post 1985	
	% of cases (N=22)[1]	% of controls (N=19)	% of cases (N=23)	% of controls (N=19)
Lamb	100	95	96	95
Pork	95	100	96	95
Beef	91	100	96	95
Venison	45	21	22	21
Veal	23	32	13	32
Poultry	100	100	100	95
Fish	100	95	91	95

[1] One case was excluded from the analysis since meat consumption prior to 1985 was not known

Almost all cases and controls had eaten lamb, pork, beef, poultry and fish since 1985. Five cases and 4 controls were reported to have eaten venison since 1985, while 3 cases and 6 controls had eaten veal. Cases were reported to consume beef more frequently than the controls (test for trend: $p = 0.002$) (Table 15). There was no evidence that cases consumed lamb, pork or fish more frequently than controls ($p > 0.10$).

Table 15 Frequency of consumption, since 1985, of beef by 23 cases of nvCJD and 19 controls

Type of meat	Frequency of consumption	% of cases (n=22)	% of controls (n=19)
Beef	< monthly	9	42
	monthly	9	26
	weekly	82	32

Most cases and controls had eaten sausages, liver, puddings, burgers and meat pies since 1985 (Table 16). About one third to a half of cases and controls were reported to have eaten kidneys, haggis and faggots (Table 16). Only a small number of cases and controls were reported to have eaten tripe (0 and 2), tongue (3 and 2), trotters (1 and 0), heart (3 and 1) since 1985. No cases or controls were reported to have ever eaten eyes, while only one control was reported to have tried "a spoonful" of brains on

one occasion, in Italy in the mid 1980s and only one control recorded as having once eaten sweetbreads (prior to 1985).

There were no striking differences between the proportions of cases and controls eating any of these items.

One route through which bovine brain and spinal cord may have entered the human food supply is through mechanically recovered meat (MRM). Data on the frequency of consumption of three of the food items listed above which might contain MRM (sausages, burgers, meat pies) were combined to provide an estimate of the frequency with which cases might have been exposed to MRM in the period since 1985 (Table 17).

Table 16 Results of a comparison between 23 cases of new variant Creutzfeldt-Jakob disease and 19 controls with regard to consumption of various animal "products"

	Ever		Post 1985	
	Number of cases (N=22)[1]	Number of controls (N=19)	Number of cases (N=23)	Number of controls (N=19)
Sausages	22	19	22	19
Liver	15	16	12	15
Kidney	10	10	8	9
Puddings	14	14	14	10
Haggis	9	7	8	6
Burgers	19 (/19)	17 (/17)	20 (/20)	15 (/17)
Meat pies	13 (/13)	11 (/13)	13 (/13)	11 (/13)
Faggots	8 (/16)	7 (/13)	6 (/17)	5 (/12)

[1] One case was excluded from the analysis since meat consumption prior to 1985 was not known

Table 17 Comparison of new variant CJD cases and controls with respect to the frequency of consumption, since 1985 of foodstuffs which might contain mechanically recovered meat (sausages, burgers, meat pies)

Frequency of consumption of sausages, burgers or meat pies	% of cases (n=20)	% of controls (n=16)
< 1 per month	5	6
several times per month	15	6
more than once per week	80	88

This analysis indicates very high rates of consumption of these food items among both cases and controls, without indicating major differences between cases and controls. The data suggest that, among the population in the age range considered (mid-teens to forties) exposure to MRM is likely to have been very widespread and have occurred on many occasions.

Occupation and new variant CJD

Table 18 presents a list of occupations for these cases and controls. No occupation stands out as unusually common among the cases.

Table 18 List of lifetime occupations for 23 cases of new variant Creutzfeldt-Jakob disease and 19 controls

Cases	Controls
Shop assistant/retail (x 7)	Shop assistant/retail (x 4)
Clerical worker (x 6)	Clerical worker (x 4)
Catering worker (x 6)	Catering worker (x 4)
Engineering (x 2)	Engineering (x 2)
Nurse (x 2)	Dental nurse
Cleaner/domestic (x 2)	Cleaner/domestic
Computing (x 2)	Hairdresser (x 2)
Horticulture (x 2)	Factory worker (x 2)
Hairdresser	Chicken factory worker
Factory worker	Management
Stable hand	Bar worker
Solicitor	Miner
Debt collector	Fireman
Bar worker	Fairground worker
Laundrette worker	Performer
Cable layer	Road layer
Forestry worker	Labourer
RAF Policeman	Bottling
Energy Industry	Driver/porter
Paper round	Painter/decorator
	Sewing Machinist
	Care Attendant
	Toolmaker

Both cases and controls had experience of a wide range of occupations, without any occupation being particularly common among cases. There is no evidence of an obvious occupational risk of nvCJD.

Summary

We have found no evidence of any iatrogenic or occupational risk of nvCJD. Although a number of cases had undergone operations so too had controls. Cases were reported to eat beef more frequently than controls. However, this finding is extremely difficult to interpret because of the problem of recall bias, previously discussed in the context of sporadic CJD. Perhaps the most striking finding is the degree of exposure of the population to food items which might contain MRM.

SECTION

4

Neuropathological Validation

Statement of Progress

The neuropathology laboratory in the CJD Unit has continued to sustain an increasing workload in terms of specimens submitted for examination (Table 19). The 4 technical staff in the laboratory (led by Mrs L. McCardle) are now all highly skilled in the particular requirements of the CJD Neuropathology Laboratory, which now acts as both a national and international reference centre. The technical staff also are responsible for the day to day running of the brain and tissue bank, and liaise closely with neuropathologists and other technical staff across the UK to provide support in organising autopsies, ensuring that diagnostic material is promptly collected and that the relevant diagnostic sections are returned to the referring pathologists whenever possible. Through these efforts, a high post-mortem rate and referral rate for suspected cases of CJD has been maintained.

Surveillance and Workload during 1996/97

Detailed breakdown of the laboratory workload is summarised in Tables 20 and 21. This demonstrates the increased workload sustained by the laboratory, as a direct consequence of the identification of the new variant form of CJD, prompted by an enormous response from clinicians and pathologists across the world who were anxious to investigate or exclude the possibility of CJD in young patients who present with undiagnosed progressive neurodegenerative disorders. The bulk of the work, however, is concerned with UK referrals directly related to the surveillance project. In this group, the largest increases are seen in cases which showed no evidence of CJD (and in whom insufficient tissue was available to provide an alternative diagnosis), along with cases of Alzheimer's disease and sporadic CJD. Fewer cases of GSS and other familial forms of CJD were identified, and the number of iatrogenic CJD cases from growth hormone recipients is maintained. Within these figures, 4 patients were referred to the laboratory on more than one occasion and were accordingly allocated multiple reference numbers for example where a brain biopsy was followed by autopsy and both sets of material were referred for examination.

Brain banking activities

The bank of frozen fixed tissues from CJD and control cases was used extensively by research collaborators both in the UK and across the world (see Table 21). This material has been particularly important in the identification of the characteristic

glycosylation pattern of PrP in the nvCJD and this important finding is currently being extended to include all cases of CJD in whom frozen material is available for analysis in collaboration with Professor John Collinge.

The increased laboratory workload is also reflected in the brain bank activities, and in the increased time spent by technical staff in organising specimen collection, transit, receipt and despatch. Our primary concern with the safe handling and transport of tissues is maintained, with no difficulties or accidents being reported this year, even in the light of the considerably increased activity in this area.

Health and Safety

The CJD laboratory has been inspected by the local Health and Safety Executive team, with satisfactory results. Attention was drawn to the need to update protocols covering the effects of major accident, e.g. fire in the neuropathology laboratory where staff might be expected to retrieve or decontaminate material in the brain store. This matter has now been resolved. The laboratory continues to receive numerous requests for advice and guidance on all aspects of the handling of CJD tissues including brain biopsy, CSF, blood samples, brain tissue specimens, autopsy procedures, burial and cremation.

Laboratory Visitors

The laboratory has been visited by neuropathologists, technical staff and scientists during the year for varying periods up to a fortnight. During this time, full training is given in all aspects of the laboratory handling, investigation and diagnosis of CJD (see Table 22).

Research Projects

The neuropathology laboratory undertakes research funded by BBSRC in the development of computerised imaging systems for the neuropathology of CJD and related disorders. The laboratory participates in a number of collaborative research projects with colleagues in the BBSRC-MRC Neuropathogenesis Unit in Edinburgh, in the Institute of Animal Health in Compton, and with the Prion Group in St. Mary's Hospital. The laboratories is also a major contributor to the EC BIOMED neuropathology project headed by Professor H. Budka and also contributes to the Biomed project for surveillance of CJD in the European Community (led by Dr R.G. Will). These activities has undoubtedly influenced the number of cases referred for investigation and opinion from Europe over the past year.

Table 19

Period 1st May 1996 – 30th April 1997
(RU96/034 – RU97/056)

STUDY CASES	**Current Year**	**Previous Year**
Animal	4	*2*
Historical	2	*7*
Kuru	7	*0*
European Community	25	*1*
Rest of World	27	*5*
Sub-total	65	*15*
SUSPECTED CASES (UK)		
No evidence of CJD*	26	*9*
Iatrogenic CJD (GHT)*	6	*5*
GSS	0	*3*
FFI	0	*0*
CJD (Classical)*	29	*23*
nvCJD*	7	*11*
Organic Dementias	6	*2*
Alzheimer's disease	15	*8*
Other†	7	*4*
TOTAL NUMBER OF CASES	**161**	**80**

† Other:

Ischaemia/hypoxia	3
Multiple infarcts	2
Tumour	1
Metabolic disorder	1

NOTES:

* In four cases, a patient has been referred to the unit on more than one occasion and has therefore been allocated multiple reference numbers.

Table 20

MATERIAL RECEIVED FOR DIAGNOSTIC CASES

Period 1st May 1996 – 30th April 1997

	Referred Cases	Studies
Fixed and frozen	34	
Fixed only	29	
Paraffin blocks	75	7
Stained slides	21	16
Unstained slides	29	16
CSF	1	
LVN	1	
TOTAL	**190**	**39**

Table 21

MATERIALS SENT FROM UNIT

Period 1st May 1996 – 30th April 1997

Name of Recipients	No of cases
Dr L Bridges, Leeds, UK	6
Dr M Bruce, Edinburgh, UK	9
Prof J Collinge, London, UK	68
Dr J Hope, Edinburgh, UK	6
Dr Kitamoto, Sendai, Japan	5
Dr Kretzschmer, Gottingen, Germany	3
Dr C Lasmezas, Paris, France	4
Prof J Lowe, Nottingham, UK	4
Dr J Manson, Edinburgh, UK	1
Prof L Manuelides, Baltimore, USA	1
Prof M Pocchiari, Rome, Italy	1
Prof S Prusiner/ Dr G Telling San Francisco, USA	6
Professor A Shapira, London, UK	1
Dr P Simmonds, Edinburgh, UK	5
Prof F Tagliavini, Milan, Italy	9
Prof B Williams, Wyoming, USA	3

Table 22

Visitors to CJD Unit Neuropathology Laboratory 1996-97

Pathologists / Neurologists / Scientists

Dr C Bergeron, Toronto, Canada

Dr D Delisle, Marseille, France

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SECTION

5

Publications

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SECTION

6

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sex for all faces by the seven subjects was near 100% (mean response over all subjects and experiments: 96.6%, range: 81.3–100%).

Image acquisition and analysis. Echoplanar MR brain images were acquired using a 1.5 Tesla GE Signa system (General Electric) retrofitted with advanced NMR hardware (ANMR) using a standard head coil. 100 T2*-weighted images depicting BOLD contrast[25] were acquired over 5 min (for each experiment) at each of 14 near-axial non-contiguous 5-mm-thick planes parallel to the intercommissural (AC-PC) line, providing whole-brain coverage: TE, 40 ms; TR, 3 s; in-plane resolution, 3 mm; interslice gap, 0.5 mm. An inversion recovery EPI dataset was also acquired at 43 near-axial 3-mm-thick planes parallel to the AC-PC line: TE, 80 ms; TI, 180 ms; TR, 16 s; in-plane resolution, 3 mm; number of signal averages, 8. The periodic change in T2*-weighted signal intensity at the (fundamental) experimentally determined frequency of alternation between A and B conditions was analysed by pseudogeneralized least-squares (PGLS) fit of a sinusoidal regression model to the movement-corrected[26] time series at each voxel, yielding parametric maps of the squared amplitude of the response at the stimulus frequency divided by its standard error—the fundamental power quotient, FPQ (ref. 27). Each observed time series was randomly permuted ten times, and FPQ estimated as above in each randomized time series, to generate 10 randomized parametric maps of FPQ for each subject in each anatomical plane. To construct generic brain activation maps, showing brain regions activated over a group of subjects, observed and randomized parametric maps of FPQ estimated in each individual were first transformed into the stereotactic space of Talairach and Tournoux and smoothed by a gaussian filter with full width at half maximum of 11 mm (ref. 28). The median observed value of FPQ was then computed at each voxel in standard space and its statistical significance tested by reference to the null distribution of median FPQ computed from the identically smoothed and spatially transformed randomized maps. For a one-tailed test of size p, the critical value was the $100^*(1-p)$th percentile of the randomization distribution[29]. To identify voxels that demonstrated significant difference in standardized power of response to faces that expressed disgust with different intensities, the observed difference in median FPQ between these two experimental conditions was computed at each voxel. Subjects were then randomly reassigned to one of two equal-sized groups and the difference in median FPQ between randomized groups was computed at each voxel[30]. This process was repeated 64 times and the results were pooled over voxels to generate a null distribution for difference in median FPQ. For a two-tailed test of size p, the critical values were the $100^*(1-p/2)$th and $100^*(p/2)$th percentiles of the randomization distribution.

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Transmissions to mice indicate that 'new variant' CJD is caused by the BSE agent

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There are many strains of the agents that cause transmissible spongiform encephalopathies (TSEs) or 'prion' diseases. These strains are distinguishable by their disease characteristics in experimentally infected animals, in particular the incubation periods and neuropathology they produce in panels of inbred mouse strains[1–4]. We have shown that the strain of agent from cattle affected by bovine spongiform encephalopathy (BSE) produces a characteristic pattern of disease in mice that is retained after experimental passage through a variety of intermediate species[5–7]. This BSE 'signature' has also been identified in transmissions to mice of TSEs of domestic cats and two exotic species of ruminant[6,8], providing the first direct evidence for the accidental spread of a TSE between species. Twenty cases of a clinically and pathologically atypical form of Creutzfeldt–Jakob disease (CJD), referred to as 'new variant' CJD (vCJD)[9], have been recognized in unusually young people in the United Kingdom, and a further case has been reported in France[10]. This has raised serious concerns that BSE may have spread to humans, putatively by dietary exposure. Here we report the interim results of transmissions of sporadic CJD and vCJD to mice. Our data provide strong evidence that the same agent strain is involved in both BSE and vCJD.

Transmissions to mice were set up from six typical sporadic cases of CJD (spCJD) and three cases of vCJD. All were homozygous for methionine at codon 129 of the 'prion protein' (PrP) gene, and none carried PrP gene mutations associated with familial disease. The spCJD cases included two dairy farmers (aged 61 and 64 years) who had had BSE in their herds and had therefore been potentially exposed to BSE-infected cattle or contaminated animal feed[11]; two 'contemporary' cases (aged 55 and 57 years) with no known occupational exposure to BSE; and two 'historical' cases (aged 57 and 82 years) who had died in 1981 and 1983, before the onset of the

BSE outbreak. All of these spCJD cases were characterized by widespread spongiform vacuolation in the brain with few or no amyloid plaques. The vCJD cases (aged 29, 30 and 31 years) had clinical and neuropathological characteristics that were atypical for CJD[9]. The main distinguishing neuropathological features in these and other vCJD cases are an extensive deposition of PrP amyloid in the brain as large 'florid' plaques and a prominent involvement of the cerebellum.

Panels of three inbred mouse strains (RIII, C57BL and VM) and one cross (C57BL × VM) were challenged with CJD brain homogenates. Previous transmissions, using the same protocol, of BSE from eight unrelated cattle (Fig. 1b) and TSEs from three domestic cats (Fig. 1c), a greater kudu and a nyala (two exotic ruminants) have given a remarkably uniform pattern of incubation periods in these mice[5,6,8]. The shortest incubation periods were seen in RIII mice, with means ranging from 302 to 335 days for transmissions from frozen brain samples. These isolates also produced strikingly similar patterns of vacuolar degeneration in the brains of infected mice, as represented by the 'lesion profile'[5,6] (Fig. 2b, c). The lesion profile is a well-established semiquantitative method of measuring the targeting of vacuolation to different brain regions, and reliably discriminates between TSE strains in mice[2]. In addition, the disease characteristics in mice injected with brain from two sheep, a goat and a pig that had been experimentally infected with BSE were very similar to those seen in direct BSE transmissions from cattle[6,7].

The BSE 'signature', based on both incubation periods and pathology, has only ever been seen in transmissions from animals suspected or known to have been infected with BSE. It has never been seen throughout an extensive series of transmissions, set up in Edinburgh between 1963 and 1994, of other naturally occurring TSEs (35 sheep and two goats with scrapie, two mink with transmissible mink encephalopathy, and a mule deer with chronic wasting disease). For example, the incubation periods and lesion profiles seen in transmissions from six sheep with scrapie, collected since 1985, are shown in Figs 1a and 2a. Within the same timescale a further two sources of sheep scrapie failed to transmit to mice. In general, natural scrapie transmissions in our own laboratory and elsewhere have given variable results, probably reflecting variation in agent strain amongst the sheep sources[12,13].

At the time of writing, the transmissions of vCJD to mice have been in progress for 360 days. The RIII mice injected with all three vCJD sources have developed a progressive clinical disease very similar to BSE, with incubation periods in individual mice ranging from 288 to 351 days. The first signs were nervousness and hypersensitivity, followed by lethargy, weight loss, urinary incontinence and postural abnormalities. Excluding early intercurrent deaths, all RIII mice injected with vCJD have developed disease, with mean incubation periods up to a standard clinical endpoint of 304 ± 4, 306 ± 6 and 310 ± 4 days (±s.e.m.) for three sources (Fig. 1d), within but at the lower end of the range previously seen for BSE and related isolates[6] (Fig. 1b, c). Clinical signs are now apparent in some of the C57BL mice, an observation that is also consistent with the BSE pattern (see Fig. 1b). Diagnosis was confirmed for all clinically affected mice by the presence of vacuolar degeneration in

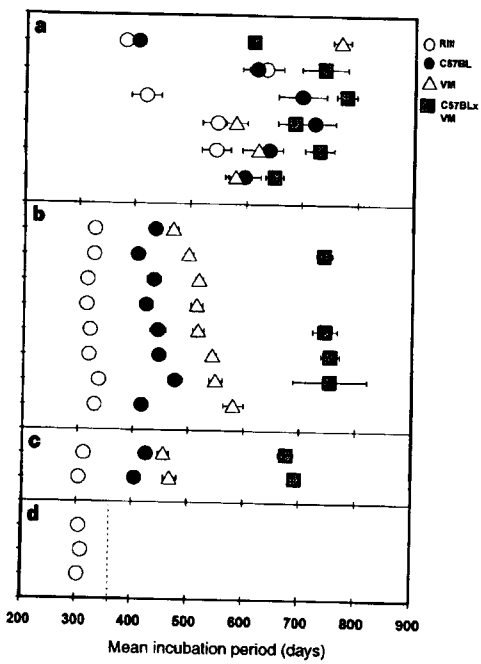

Figure 1 Incubation times in mice with spongiform encephalopathies. Incubation periods in RIII, C57BL, VM and C57BL × VM mice in transmissions of: **a**, natural scrapie from six sheep; **b**, BSE from eight cattle; **c**, FSE from two cats; and **d**, three cases of vCJD. C57BL × VM mice were not included in the first, third and fourth BSE transmission; missing symbols elsewhere indicate that no clinical disease was seen in these groups up to the natural lifespan of the mice. The vertical dotted line in **d** shows the current time after challenge in experiments still in progress. Data are mean ± s.e.m.

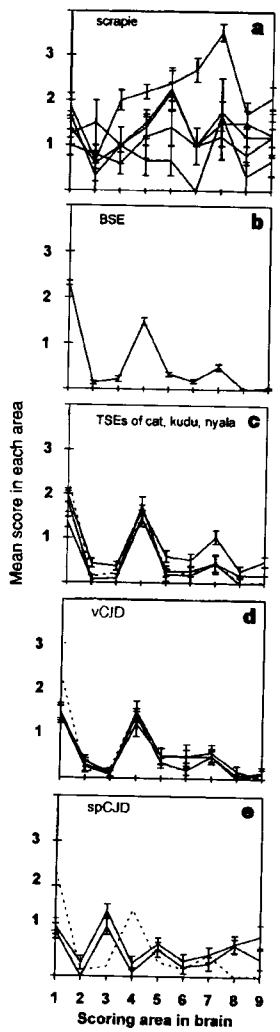

Figure 2 Lesion profiles for mice following transmission of spongiform encephalopathies. Lesion profiles are for RIII mice in transmissions of: **a**, natural scrapie from five of the six sheep from Fig. 1a (n = 3–16 mice per group; no clinical disease was seen in this mouse strain in the sixth transmission); **b**, BSE from the first four cattle (n = 123, pooled data); **c**, FSE from two cats (n = 36, pooled data) and TSEs from a greater kudu and a nyala (n = 12 and 11); **d**, vCJD from three sources (n = 10, 12 and 16) and **e**, spCJD from two sources, a farmer and a contemporary case (n = 8 and 9). The pooled BSE profile is shown as a dotted line in **c**–**e**. As white-matter vacuolation was not a prominent feature in any of these transmissions, only the grey-matter lesion profiles are shown. Vacuolation was scored on a scale of 0–5 in the following scoring areas: 1, dorsal medulla; 2, cerebellar cortex; 3, superior colliculus; 4, hypothalamus; 5, thalamus; 6, hippocampus; 7, septum; 8, retrosplenial and adjacent motor cortex; and 9, cingulate and adjacent motor cortex. Data are mean ± s.e.m.

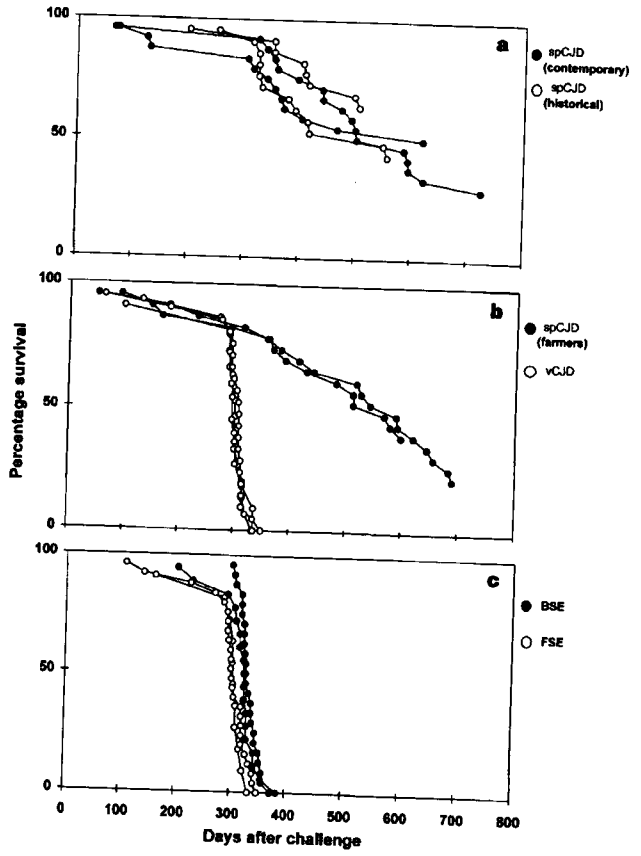

Figure 3 Survival curves for mice following transmission of spongiform encephalopathies. Survival curves are for female RIII mice in transmissions of: **a**, spCJD from cases with no known occupational exposure to BSE; **b**, spCJD from two farmers and vCJD from three sources; and **c**, BSE from the first two cattle sources and FSE from two cats. No distinction is made between mice dying with clinical signs of TSE infection and mice dying with intercurrent disease. Deaths up to 50 days after challenge, most of which were related to injection trauma, are excluded from the analysis.

the brain and for selected mice in all three experiments by the demonstration of relatively protease-resistant isoforms of PrP (PrP^{res}) in western blots of brain extracts and pathological accumulations of PrP in immunostained brain sections.

The neuropathology in clinically affected RIII mice with vCJD was also similar to that seen in RIII mice with BSE, consisting of a mild-to-moderate grey-matter vacuolation of the hypothalamus, medulla oblongata and septum, and a more severe vacuolation of the cochlear nucleus. Amyloid plaques were not a prominent feature of this pathology. The lesion profiles in RIII mice for the three sources (Fig. 2d) were very similar to each other and also to those in transmissions to RIII mice of BSE (Fig. 2b), TSEs of cats and exotic ruminants (Fig. 2c), and experimental sheep, goat and pig BSE[6,7], but differed markedly from those seen in transmissions from sheep with natural scrapie (Fig. 2a). Although results are so far only available for the RIII mouse strain, the striking similarity between vCJD and BSE in these mice, in terms of both incubation periods and pathology, is in itself strong evidence that the same strain of agent is involved in vCJD and BSE.

In contrast to the results with the vCJD sources, no clinical signs of neurological disease have yet been seen in any mice in the six transmissions of spCJD, although they have been in progress for between 600 and 800 days. Figure 3 shows survival curves for RIII mice in these experiments, compared with survival curves in BSE, feline spongiform encephalopathy (FSE) and vCJD transmissions. No significant differences in median survival times were found between RIII groups challenged with the six spCJD sources, or between these groups and saline-injected controls. However, this does not indicate a failure to transmit spCJD, as vacuolar degeneration typical of TSE infection was seen in the brains of some mice dying with intercurrent disease in all six experiments, from about 400 days after challenge. This pathology has so far been seen in 130 of the 156 mice surviving beyond 500 days after injection for which brain was available for histopathological scrutiny. No such changes have been seen in the control mice of any age in this set of experiments, or in mice of the same strains injected with human brain homogenates from patients with amyotrophic lateral sclerosis or laryngeal carcinoma in a previously study[14]. Western blot and immunohistochemical analyses have demonstrated the accumulation of PrP^{res} in selected brains showing vacuolar pathology, confirming successful transmission of a TSE from all six spCJD sources.

A full analysis of the pathology in recipient mice in spCJD transmissions will be presented when these experiments are complete, but already several points can be made. Vacuolar degeneration has been seen in all four mouse strains. This pathology differs in severity between individual mice, but shows a consistent pattern between mouse strains and between spCJD sources. The earliest pathology is seen consistently in the superior colliculus and olfactory tract. In brains showing more widespread vacuolation there is also prominent involvement of the cerebral cortex, thalamus, hypothalamus, caudate nucleus and optic tract, a distribution unlike that seen in BSE transmissions to mice. As an illustration, Fig. 2e shows the lesion profiles for RIII mice killed with intercurrent disease or culled between 500 and 750 days after challenge with two spCJD sources (a farmer and a contemporary case). Although these profiles are not based on animals at the clinical endpoint of the disease, they clearly show a similarity in lesion distribution between the two sources of spCJD, and a difference between these sources and vCJD or BSE, particularly in scoring area 3, the superior colliculus. The results of these transmissions therefore provide no evidence of a link between CJD in dairy farmers and BSE.

A series of transmissions to mice of spCJD and familial human TSEs associated with mutations in the PrP gene have been reported in Japan[15]. Although different mouse strains were used in the Japanese series, the results for transmissions of spCJD from 129-methionine sources were broadly similar to ours in that transmission was achieved from all sources and mean incubation periods in recipient mice were long (573–863 days)[15]. Transmissions of the familial TSE Gerstmann–Straussler–Scheinker syndrome (GSS) were achieved from only one-third of the sources tested, but the mean incubation periods in successful transmissions were relatively short (237–517 days)[15]. Although some of these incubation periods were quite close to our results for vCJD in RIII mice, the pathology in mice with GSS was strikingly different as it included a prominent vacuolation of white-matter tracts[16]. The Japanese workers also reported the transmission of another human familial TSE, fatal familial insomnia (FFI), with a mean incubation period of 455 days in recipient mice[17]. The pathology in mice with FFI was indistinguishable from that in mice with spCJD in the Japanese series, apart from there being a more pronounced involvement of the thalamus.

Our results highlight several fundamental features of the TSEs previously established using experimental isolates[3,4]. The consistency in transmission properties shows that the agent must interact with genetic factors in the host to control the timing and neuropathology of the disease with extraordinary precision. Different strains of agent (spCJD, vCJD) can be isolated from hosts with the same PrP amino-acid sequence (in this case, patients with the 129-methionine genotype) but, conversely, the same strain of agent can

be detected in hosts with different PrP sequences (so far the BSE 'signature' has been seen in transmissions from eight different species[6]). This clearly indicates that TSE agents carry some form of information that specifies strain-specific properties, but the molecular basis of this information is still a matter for speculation[4].

It has been reported that vCJD can be distinguished from spCJD by the relative prominence of differently glycosylated forms of PrP^{res} and the molecular size of the unglycosylated form[18]. Samples from dairy farmers with CJD have given glycoform ratios resembling those from other cases of spCJD[19]. A similarity in glycoform patterns between vCJD and BSE has been presented as evidence of a link between the two[18]. However, a 'BSE-like' glycoform pattern has also been seen for experimental scrapie isolates that are unrelated to BSE[20] and for FFI in humans[21]. Therefore, although the analysis of PrP diversity provides a useful supplement to strain typing in mice, it is premature to draw conclusions concerning causative links between TSEs in different species on the basis of glycoform-ratio analysis alone. A full analysis of glycoform patterns in the present series of transmissions will be reported in due course.

In conclusion, strain typing based on transmission to mice has shown: that vCJD is caused by the same strain of agent that has caused BSE, FSE and TSEs in exotic ruminants; that vCJD is distinguishable from spCJD; and that CJD in two dairy farmers is of the spCJD type and is not linked to the causative agent of BSE. Epidemiological surveillance continues to indicate that vCJD is a new condition occurring almost exclusively in the UK. Our transmission studies, in combination with the surveillance data, provide compelling evidence of a link between BSE and vCJD. □

Methods

CJD inocula. The CJD challenge experiments were the first to be undertaken within a new category 3 containment facility at the Neuropathogenesis Unit in Edinburgh, in an environment in which no TSE-infected materials had been handled previously. New dedicated glassware and instruments were autoclaved at 136 °C for 1 h before use. Brain samples for transmission were collected, as far as possible, from areas showing maximum pathology, and stored at −20 °C. Samples were homogenized at 10% (w/v) concentration in sterile physiological saline and stored at −20 °C. Before homogenization of each sample, sterile physiological saline was run through the homogenizer and other glassware and frozen for later inoculation of the appropriate control group. For injection, thawed homogenates were resuspended by being drawn repeatedly through a series of graded needles.

CJD transmissions. Three inbred mouse strains and one cross were challenged: C57BL and RIII (both of the *Sinc*^{s7} or *Prn-p*^{a} genotype), VM (*Sinc*^{p7} or *Prn-p*^{b} genotype), and the F_1 cross between C57BL and VM[22,23]. Groups of approximately 20 mice of each strain were injected by a combination of the intracerebral (20 μl) and intraperitoneal (100 μl) routes under halothane anaesthesia. For each transmission, six mice of each strain were injected with the appropriate saline sample by the same routes. Groups of uninjected control mice were also included. Mice were coded, examined daily throughout their lifespan, and formally scored for signs of neurological disease from 250 days after injection. Mice showing definite signs for two consecutive weeks were killed and incubation periods calculated as the interval between injection and this standard clinical endpoint[22]. All other mice were maintained to full lifespan, apart from small numbers culled at 700–750 days post-injection, to avoid loss of pathological material.

Histopathological and protein analysis. At post-mortem, a lateral third of each mouse brain was dissected aseptically and frozen at −20 °C for protein analysis and further passage. The remaining two-thirds of each brain was immersion fixed in 10% formol saline for 4 days, treated with 98–100% formic acid for 1 h to inactivate infectivity, and fixed in formol saline for a further 2 days. The brains were trimmed at standard coronal levels and paraffin embedded. Haematoxylin and eosin-stained sections 6 μm thick were prepared, randomly mixed with others from BSE, FSE, sheep scrapie and mouse-passaged scrapie transmissions, and coded for pathological assessment. Vacuolar changes were scored in nine grey-matter and three white-matter areas of brain for the construction of lesion profiles, as described[24]. PrP in brain sections was immunostained using a polyclonal antibody to mouse PrP, 1A8 (ref. 25), according to a published protocol[26]. SDS–polyacrylamide gel electrophoresis and western blot analysis of brain tissue were used to confirm the presence of PrP^{res} (ref. 18).

Animal TSE transmissions. The CJD transmissions were compared with transmissions of TSEs from cattle, sheep, domestic cats, a greater kudu and a nyala; the results of some of these animal TSE transmissions have been included in previous publications[5,6,8]. The design of these experiments was identical to that in the CJD transmissions, except that the source material from one cat, the greater kudu and the nyala was formol-fixed brain. Because transmissions from fixed tissues have resulted in prolonged incubation periods[5], probably owing to loss of titre, the incubation period and survival data from these three transmissions are not included in Figs 1 and 3. However, as the pathology in experimentally infected mice is unaffected by the dose of TSE challenge, lesion profile data from the kudu and nyala transmissions are included in Fig. 2.

Statistical analyses. Statistical analyses were performed using the software package Stata, version 5.0. Kaplan–Meier survival curves were plotted and differences in survival between mice inoculated with material from different sources were compared using the log-rank test[27]. Principal components analysis[28] was used to calculate summary measures of lesion profiles and to examine graphically the 'closeness' of the lesion profiles from different transmissions. This statistical analysis was in complete agreement with the subjective judgement of 'closeness' described in the text and will be documented in detail in a future publication.

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