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A case cluster of variant Creutzfeldt-Jakob disease linked to the Kingdom of Saudi Arabia

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Abstract

As of mid-2016, 231 cases of variant Creutzfeldt-Jakob disease—the human form of a prion disease of cattle, bovine spongiform encephalopathy—have been reported from 12 countries. With few exceptions, the affected individuals had histories of extended residence in the UK or other Western European countries during the period (1980–96) of maximum global risk for human exposure to bovine spongiform encephalopathy. However, the possibility remains that other

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Supplementary material

Supplementary material is available at *Brain* online.

geographic foci of human infection exist, identification of which may help to foreshadow the future of the epidemic. We report results of a quantitative analysis of country-specific relative risks of infection for three individuals diagnosed with variant Creutzfeldt-Jakob disease in the USA and Canada. All were born and raised in Saudi Arabia, but had histories of residence and travel in other countries. To calculate country-specific relative probabilities of infection, we aligned each patient's life history with published estimates of probability distributions of incubation period and age at infection parameters from a UK cohort of 171 variant Creutzfeldt-Jakob disease cases. The distributions were then partitioned into probability density fractions according to time intervals of the patient's residence and travel history, and the density fractions were combined by country. This calculation was performed for incubation period alone, age at infection alone, and jointly for incubation and age at infection. Country-specific fractions were normalized either to the total density between the individual's dates of birth and symptom onset ('lifetime'), or to that between 1980 and 1996, for a total of six combinations of parameter and interval. The country-specific relative probability of infection for Saudi Arabia clearly ranked highest under each of the six combinations of parameter × interval for Patients 1 and 2, with values ranging from 0.572 to 0.998, respectively, for Patient 2 (age at infection × lifetime) and Patient 1 (joint incubation and age at infection × 1980–96). For Patient 3, relative probabilities for Saudi Arabia were not as distinct from those for other countries using the lifetime interval: 0.394, 0.360 and 0.378, respectively, for incubation period, age at infection and jointly for incubation and age at infection. However, for this patient Saudi Arabia clearly ranked highest within the 1980–96 period: 0.859, 0.871 and 0.865, respectively, for incubation period, age at infection and jointly for incubation and age at infection. These findings support the hypothesis that human infection with bovine spongiform encephalopathy occurred in Saudi Arabia.

Keywords

prion diseases; variant Creutzfeldt-Jakob disease; bovine spongiform encephalopathy; Saudi Arabia

Introduction

Human prion diseases are rare, fatal, incurable neurodegenerative disorders that typically cause about 1–2 deaths per million population per year (Ladogana *et al.*, 2005; Holman *et al.*, 2010; Coulthart *et al.*, 2015). Prion diseases are caused by pathological misfolding and aggregation of a cellular glycoprotein, the prion protein (PrP, encoded by *PRNP*). Unlike other neurodegenerative diseases entailing protein misfolding, prion diseases are transmissible between individuals because the pathologic form of PrP (PrP^{Sc}) promotes misfolding of normal PrP. The term 'prion' denotes the aetiological proteinaceous infectious particle (Prusiner, 1998).

Variant Creutzfeldt-Jakob disease (vCJD) is a human prion disease linked to a prion disease of cattle, bovine spongiform encephalopathy (BSE), which was identified in the UK during the 1980s and subsequently spread internationally (Will *et al.*, 1996; Diack *et al.*, 2014). Between 1994 and 2016, 231 cases of vCJD have been identified in residents of 12 countries [The National CJD Research and Surveillance Unit (NCJDRSU), 2016]. Of these patients,

222 were residents of the UK and other European countries where BSE was most prevalent. Among the other nine cases, four were linked to residence in the UK between 1980 and 1996 when BSE exposure risk was highest there and globally. One additional case was attributed to a brief UK visit in 1990, and another most likely to residence for more than 6 years each in Kuwait or Russia between 1980 and 1996 (Jansen *et al.*, 2003; Yamada and Variant CJD Working Group, Creutzfeldt-Jakob Disease Surveillance Committee, Japan, 2006; Holman *et al.*, 2010; Yang *et al.*, 2010; Maheshwari *et al.*, 2015).

In this Report, we present diagnostic and epidemiological findings for the remaining three vCJD patients, and conclude they were most likely infected while residing in the Kingdom of Saudi Arabia.

Materials and methods

Patients, diagnoses and histories

Narrative accounts of patient presentation, disease course, supporting investigations and final diagnoses are provided in the Supplementary material. Lifetime residence and travel histories were obtained through interviews with family members and from additional documents such as passports.

Statistical analysis

Country-specific probabilities of infection were estimated for each case by aligning each patient's lifetime histories with gamma distributions of incubation period and age at infection, using dates of symptom onset and birth, respectively, as anchor points. Fractions of probability density within each life interval were then computed, normalizing the density within an interval to the total density between birth and onset. Fractions were combined by country, to give country-specific fractions summing to 1 for each patient. Gamma distributions were based on modelling of the cohort of 171 vCJD cases to 2009 in the UK: medians of 11.6 years and 17.9 years for incubation period and age at infection, respectively (Garske and Ghani, 2010). Note that this published study used date of death and not date of onset as the reference point for incubation period. We therefore subtracted a constant (median vCJD disease duration, 14 months) from the above-noted 11.6 years, to yield 10.4 years (Garske and Ghani, 2010; Heath *et al.*, 2010). To estimate 95% credibility intervals (CrIs) of the country-specific probability fractions, we used 2000 lines of simulation output, kindly shared by Dr Tini Garske and Dr Azra Ghani (Garske and Ghani, 2010), by re-computing probability fractions with parameter values in each line of output. CrI bounds were defined as the 2.5th and 97.5th percentile values in the sorted list of resulting fractions.

We also calculated country-specific probability fractions normalized to the time interval 1980–96 of maximum global risk for human exposure to BSE (Smith and Bradley, 2003). Lastly, we estimated joint median probability fractions and 95% CrIs for incubation period and age at infection, using the respective means of the iterations described above.

The R code used for the above analyses is available at: https://gist.github.com/alexdemarsh/38ce1583f1633bc7a6b4.

Results

Table 1 summarizes the key findings supporting two definite (Patients 1 and 2) and one probable (Patient 3) diagnoses of vCJD. None of the findings was atypical with reference to standard vCJD case-definition criteria. All diagnoses were supported by tissue pathology. Figure 1 displays key diagnostic findings from MRI and pathology.

Figure 2 summarizes the patients' lifetime histories of residence and travel. Patient 1 resided in Saudi Arabia continuously from birth in 1970 until 1997, then spent approximately 1 year in the USA before returning to Saudi Arabia in late 1998, where he remained until onset of illness in early 2003. There were no extended periods of residence in other countries, although brief visits were made to the UK (late 1997, 4 days) and France (1995, 2 weeks). He had eaten a variety of meats and meat products during the 1980s, including beef.

Patient 2 resided continuously in Saudi Arabia from birth in 1983 until 1999, and then spent ~6 years in Egypt before moving to the USA in late 2005, where he remained until onset of illness in early 2006. Travel included annual visits, 1–1.5 months in duration, to Egypt between 1989 and 1999. There had been several brief visits to the USA between 1989 and 2004, but no history of residence or travel in the UK or Europe. He had consumed beef regularly.

Patient 3 resided continuously in Saudi Arabia from birth in 1986 until 1999, and then migrated to the UAE where he resided until 2003. After this, he spent ~4 years as a resident of Bangladesh, 2 years in the USA, then 4 months in the UAE before migrating to Canada in early 2010, very shortly after onset of illness. Travel included visits ranging from 1 week to 2 months in duration to the UK, France, Canada and Bangladesh between 1986 and 2010. The UK travel consisted of three brief visits, one in late 1995 (2 weeks) and two in 2009 (total 4 weeks). Travel to France was for 1 week in late 2002, during which no beef was consumed. He consumed beef regularly during his life, including traditional dishes containing bovine brain.

Table 2 summarizes, for each patient, the estimated relative probabilities of infection in different countries, calculated as described in the 'Materials and methods' section. For Patient 1, by far the single largest country-specific probability density fractions ($\geqslant 0.98$) were linked to periods of residence in Saudi Arabia. This result was observed with incubation- and age-based probability fractions individually as well as jointly, and with fractions normalized to both the patient's total lifetime and the interval 1980–96. For Patient 2 the results showed a lower weighting toward Saudi Arabia for the lifetime age-based and joint density fractions; i.e. 0.572 [(95% CrI: 0.461, 0.685) and 0.715 (0.659, 0.772), respectively]. However, for this patient as well, Saudi Arabia ranked highest under all parameter × interval combinations.

In the case of Patient 3, whose residence and travel history was more complex than those of Patients 1 and 2, interpretation was somewhat less clear-cut, for example with lifetime incubation-based probability density fractions of 0.394 (0.226, 0.551) and 0.472 (0.321, 0.645) for Saudi Arabia and the UAE, respectively. However, again Saudi Arabia emerged as the country with the single largest point estimates with reference to the 1980–96 period; for

example, 0.859 (0.858, 0.870) for the incubation-based estimate. Joint estimates based on both incubation and age distributions were consistent with this pattern for all three patients.

Discussion

We have presented clinical and diagnostic findings for patients with vCJD, including results of EEG, MRI, CSF protein marker assay, molecular genetic analysis, PrP immunoblot, and histopathological examination of brain and/or lymphoid tissue using PrP immunohistochemistry. All patients met established criteria according to internationally accepted vCJD case definitions. The diagnoses were supported by multiple forms of evidence, in two cases with neuropathology (final diagnosis: definite vCJD) and one with tonsil pathology (final diagnosis: probable vCJD). We also documented lifetime residence and travel histories for each patient. These were used to estimate relative probabilities of infectious exposure in the various countries in which each patient had resided or travelled, using distributions of incubation period and age at infection derived from probabilistic modelling of the best-studied cohort from the UK vCJD epidemic (Garske and Ghani, 2010). Our conclusion in each case was that the single most probable country in which infectious exposure took place was Saudi Arabia.

There is compelling evidence that BSE is the zoonotic cause of vCJD (Will *et al.*, 1996; Bruce *et al.*, 1997; Hill *et al.*, 1997; Scott *et al.*, 1999; Diack *et al.*, 2014). Exposure to BSE-contaminated UK beef products between 1980, when epidemic transmission of BSE is estimated to have begun in the UK, and 1996 when reinforced regulation of animal feed was implemented there, is considered the major global risk factor for vCJD (Smith and Bradley, 2003). During this period nearly 170 000 cases of BSE were confirmed in the UK, over 28 times the total number of such cases reported in the rest of the world through 2014 [World Organization for Animal Health (OIE), 2014]. It is estimated that 1–3 million UK cattle were infected with BSE, of which most were undetected and processed for human consumption (Anderson *et al.*, 1996; Donnelly *et al.*, 2002). This is reflected in the fact that 178 of the 231 vCJD cases reported worldwide to date have occurred in UK residents (NCJDRSU, UK). For countries reporting vCJD cases not linked to UK residence, a significant correlation has been demonstrated between the number of vCJD cases and the volume of UK beef imported between 1980 and 1996 (Sanchez-Juan *et al.*, 2007). Even in France, with over 1000 BSE cases and 27 vCJD cases reported as of 2015, imported UK beef was a major source of exposure to BSE (Chadeau-Hyam and Alperovitch, 2005).

For the same reasons, it is not surprising that four of the nine patients with vCJD who resided in countries outside the UK and Western Europe at time of onset—including three of the seven who became ill and/or were diagnosed in Canada and the USA—had resided in the UK for extended periods between 1980 and 1996 (Jansen *et al.*, 2003; Holman *et al.*, 2010; Yang *et al.*, 2010). A fifth case, the only one reported to date in a Japanese resident, was attributed to exposure during a 24-day visit to the UK in early 1990, close to the peak of the human BSE exposure risk there (Yamada and Variant CJD Working Group, Creutzfeldt-Jakob Disease Surveillance Committee, Japan, 2006). The absence of other vCJD cases in Japan supports this interpretation; however, this is the only case to date in which infectious exposure has been attributed to such a brief period outside the patient's main country of

residence (Yamada and Variant CJD Working Group, Creutzfeldt-Jakob Disease Surveillance Committee, Japan, 2006).

In contrast with this general pattern, the three patients reported here were all born and raised in Saudi Arabia, and resided there for at least 10 years during the period (1980–96) of maximal global exposure risk to BSE-contaminated UK beef. None of the three had ever resided in the UK; two had not visited there between 1980 and 1996; and the third had visited there for 2 weeks in late 1995. These features of the patients' histories alone suggest that their infectious exposures more likely occurred in Saudi Arabia than in the UK or Europe. The results of our analysis of country-specific relative probabilities of infectious exposure strongly support this conclusion. In all cases—even for Patient 3, who spent the most time outside Saudi Arabia—the single greatest weight of probability for country of infection was allocated to Saudi Arabia.

Consistent with this interpretation are UK Customs and Excise data indicating that over 19 000 tons of bovine carcass meat were exported to Saudi Arabia in the period 1980–96 inclusively, with >2900 tons from 1988–90, and >2580 tons during the period 1993–96. Only relatively small amounts (<8 tons) of UK carcass meat were recorded as shipped during 1991–1992, likely reflecting a 1990 embargo applied by Saudi Arabia (Her Majesty's Revenue and Customs, UK, personal communication). Furthermore, given the life-history data (Fig. 2), even short-term importation of UK beef products to Saudi Arabia (e.g. in 1989 or 1990) could have been sufficient for all three patients to be infected. We note that, to justify the epidemiological inference that human exposure to BSE has occurred in Saudi Arabia, it would be sufficient to demonstrate this point for any individual case.

Questions could be raised regarding the realism of the country-specific probability weightings we have estimated. For example, despite our use of incubation period and age at infection estimates from the largest, most recent and best-studied cohort of UK vCJD cases, these estimates arguably may not be directly applicable to non-UK populations (Garske and Ghani, 2010). A related point has to do with the fact that earlier studies had yielded different values (e.g. incubation periods in excess of 16 years)—presumably consistent with the relatively late dates of onset of the three patients described here (2003, 2006 and 2010) (Valleron *et al.*, 2001; Boelle *et al.*, 2003).

To address these questions, we first point out that there is no specific evidence to support such epidemiological differences and that, as noted above, all three patients described here displayed clinical, paraclinical and pathological features typical of vCJD cases reported from the UK and other countries (Heath *et al.*, 2010). As also noted above, the risk of exposure to BSE-contaminated beef is considered to have applied globally, and not only in the UK, suggesting that in a broad sense all cases of vCJD can be considered to be part of the same epidemic. Lastly, to address the possibility that a longer incubation period would have a significant effect on the results of our analysis, we repeated the above computations using a mean incubation period of 16.7 years (Valleron *et al.*, 2001). Although we found small differences in some country-specific probabilities (for Patient 3 only), the key conclusions were unchanged (results not shown).

The limited number of clinical cases of vCJD confirmed worldwide to date, with only one vCJD case reported in 2013 (in the UK), one in 2014 (in the USA), and two in 2016 (one each in the UK and Italy) supports an optimistic view that few additional vCJD deaths are likely. Nevertheless, uncertainty remains regarding size and duration of the rightward 'tail' of the epidemic curve (Garske and Ghani, 2010). This uncertainty is accentuated by the results of a laboratory-based survey of archived tonsil and appendix specimens, which estimated a subclinical vCJD infection prevalence of 493 per million (95% confidence interval: 282, 801) in the general UK population, much higher than suggested by the number of clinical vCJD cases reported in that country to date (Gill *et al.*, 2013).

This tissue-based survey also demonstrated that even though almost all vCJD patients examined to date, including the three reported here, have been homozygous for the ATG (methionine) allele at codon 129 of the *PRNP* gene, individuals with any of the three possible genotypes at this codon can be infected by the BSE/vCJD agent. The potential epidemiologic significance of this genetic risk factor has been further highlighted by a recent report from the UK of an autopsy-confirmed case of vCJD in an individual who was heterozygous for ATG (methionine) and GTG (valine) alleles at *PRNP* codon 129 (Will *et al.*, 2016). This finding is consistent with the hypothesis that future cases of vCJD may occur in individuals with longer incubation periods who are heterozygous for methionine and valine alleles at *PRNP* codon 129, or possibly homozygous for the valine allele (Garske and Ghani, 2010).

We also note that Patient 3, who was heterozygous for GAG (glutamic acid) and AAG (lysine) alleles at *PRNP* codon 219, is the third vCJD patient with this genotype reported to date (Lukic *et al.*, 2010). This genotype is relatively common in Eastern and Southern Asia and the Pacific, where it reaches population frequencies in the range of 1–10% and is deemed to be protective against sporadic CJD, but very rare elsewhere (Shibuya *et al.*, 1998; Jeong *et al.*, 2005; Soldevila *et al.*, 2006). Heterozygosity at codon 219 has also been proposed to increase susceptibility to vCJD, suggesting that the likelihood of future vCJD cases occurring in residents of non-European countries may be influenced by this genetic factor (Lukic *et al.*, 2010). With the small number of such cases reported to date, however, and noting that our Patient 3 was of South Asian background, further investigation of this important hypothesis is warranted.

Still other questions relate to atypical forms of BSE that appear to arise spontaneously in cattle, as sporadic CJD is believed to originate in humans. It is presently unknown whether atypical BSE can cause human disease, or whether the original BSE outbreak emerged in this way (Comoy *et al.*, 2008; Tranulis *et al.*, 2011). These uncertainties, as well as the prolonged incubation period of vCJD and strong evidence of its transmissibility by blood transfusion, support ongoing precaution. Current measures to monitor and control BSE and CJD internationally remain key elements of a prudent long-term public health strateg (Diack *et al.*, 2014).

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Abbreviations

BSE	bovine spongiform encephalopathy
(v)CJD	(variant) Creutzfeldt-Jakob disease
95% CrI	95% credible interval

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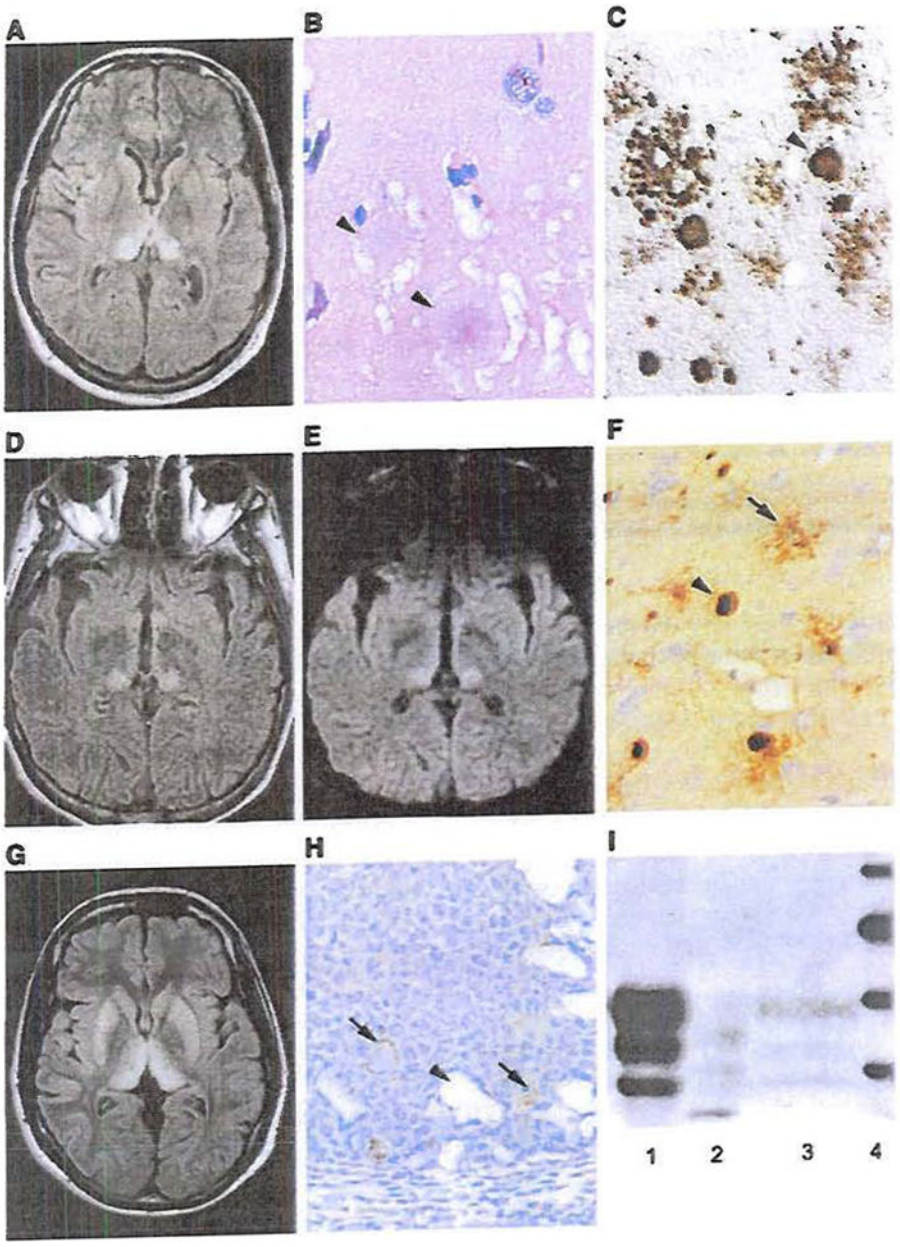

Figure 1. MRI images and pathology of Patients 1–3

(**A**) Patient 1, fluid-attenuated inversion recovery (FLAIR) image with pulvinar sign. (**B**) Patient 1, histology of brain biopsy (haematoxylin and eosin stain) showing two florid plaques (arrowheads). (**C**) Patient 1, prion protein immunohistochemistry (3F4 antibody) with astrocytes and florid plaque core staining (arrowhead). (**D**) Patient 2, FLAIR image with pulvinar sign. (**E**) Patient 2, diffusion-weighted image. (**F**) Patient 2, prion protein immunohistochemistry (3F4 antibody) showing plaque core staining (arrowhead) and astrocytic profile staining (arrow). (**G**) Patient 3, FLAIR image with pulvinar sign. (**H**) Patient 3, prion protein immunohistochemistry of tonsil (12F10 antibody) showing follicle with stained follicular dendritic cells (arrows) and artefact (arrowhead) due to accidental freezing during specimen transportation. (**I**) Patient 3, western immunoblot (3F4 antibody)

showing proteinase K-resistant prion protein isoforms: lane 1, vCJD brain tissue (type 2B); lane 2, sporadic CJD brain tissue (type 1); lane 3, tonsil tissue, showing similar pattern to lane 1; lane 4. molecular size markers with 20, 30, 40 and 50 kDa bands shown (from *bottom* to *top* of image).

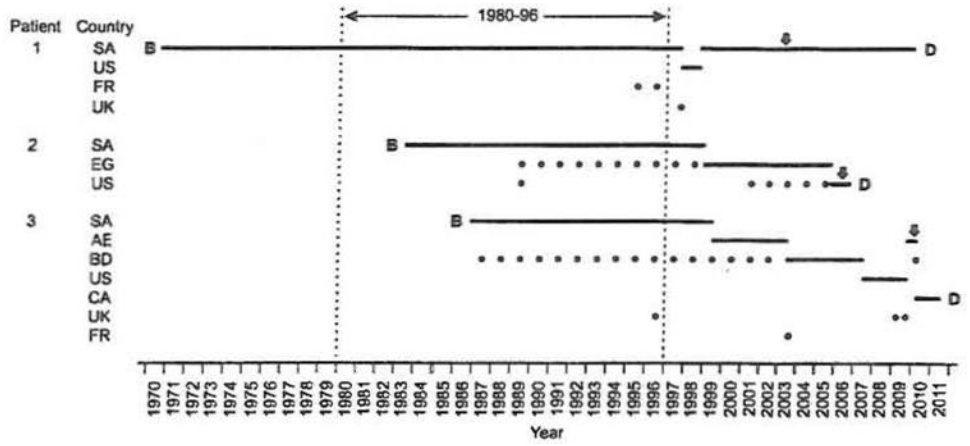

Figure 2. Residence and travel histories for the vCJD patients

Periods of residence in different countries are illustrated as horizontal bars; dates of shorter visits as dots. Dates of birth and death are indicated by ‘B’ and ‘D’, respectively. Dates of onset are marked with solid vertical arrows. Country codes are listed at left, as per ISO 3166-1 alpha-2 standard: AE = Arab Emirates; BD = Bangladesh; CA = Canada; EG = Egypt; FR = France; SA = Saudi Arabia; UK = United Kingdom; US = United States of America. The period of maximal global human BSE exposure risk from UK beef (1980–96) is indicated by vertical dotted lines.

Table 1

Key diagnostic features of the vCJD patients

	Patient		
Investigation	**1**	**2**	**3**
Age at onset	33 years	23 years	23 years
Disease duration	83 months	8.5 months	18 months
Electroencephalography	Slowing; no PSWCs	Slowing; intermittent PSWCs	Slowing: no PSWCs
CSF 14-3-3	Negative	Negative	Negative
PRNP sequencing	129M/M; no mutations	129M/M; no mutations	129M/M; 219E/K; no mutations
MRI brain	Pulvinar sign present	Pulvinar sign present	Pulvinar sign present
Pathology: brain	Positive	Positive	ND
Pathology: lymphoid	ND	Positive (PrPSc)	Positive (PrPSc)
PrP-res Immunoblot: brain	Positive	ND	ND
PrP-res Immunoblot tonsil	ND	ND	Positive
Final diagnosis	Definite vCJD	Definite vCJD	Probable vCJD

PSWC = periodic triphasic sharp-wave complexes at ~1 Hz; CSF 14-3-3 = immunoblotting assay for 14-3-3 protein in CSF; 129M/M = homozygous for methionine-encoding (ATG) DNA sequence at *PRNP* codon 129; 219E/K = heterozoygous for glutamic acid-encoding (GAG) and lysine-encoding (AAG) DNA sequences at *PRNP* codon 219; PrP^{Sc} = disease-associated prion protein; PrP-res = protease-resistant prion protein; ND = not done.

Table 2

Fractions of probability density for BSE exposure in different countries, based on incubation period and age at exposure

Patient	Country[a]	Incubation: lifetime[b,c]	Incubation: 1980–96[b,d]	Age: lifetime[b,c]	Age: 1980–96[b,d]	Joint: lifetime[b,c]	Joint: 1980–96[b,d]
1	FR	0.003 (0.001, 0.006)	0.003 (0.001, 0.006)	0.001 (0.001, 0.001)	0.001 (0.001, 0.002)	0.002 (0.001, 0.004)	0.002 (0.001, 0.004)
	GB	0.000 (0.000, 0.000)	—	0.000 (0.000, 0.000)	—	0.000 (0.000, 0.000)	—
	SA	0.996 (0.987, 0.999)	0.997 (0.994, 0.999)	0.979 (0.971, 0.988)	0.999 (0.998, 0.999)	0.988 (0.982, 0.993)	0.998 (0.996, 0.999)
	US	0.000 (0.000, 0.007)	—	0.019 (0.011, 0.027)	—	0.010 (0.005, 0.015)	—
2	EG	0.136 (0.131, 0.184)	0.138 (0.133, 0.143)	0.351 (0.257, 0.437)	0.085 (0.077, 0.092)	0.247 (0.198, 0.291)	0.111 (0.107, 0.116)
	SA	0.864 (0.814, 0.869)	0.862 (0.857, 0.865)	0.572 (0.461, 0.685)	0.909 (0.899, 0.922)	0.715 (0.659, 0.772)	0.886 (0.880, 0.892)
	US	0.000 (0.000, 0.002)	0.000 (0.000, 0.003)	0.077 (0.057, 0.103)	0.005 (0.001, 0.012)	0.039 (0.029, 0.052)	0.003 (0.001, 0.006)
3	AE	0.472 (0.321, 0.645)	—	0.241 (0.170, 0.297)	—	0.356 (0.269, 0.454)	—
	BD	0.126 (0.123, 0.168)	0.126 (0.120, 0.127)	0.298 (0.242, 0.360)	0.121 (0.119, 0.123)	0.215 (0.185, 0.247)	0.124 (0.120, 0.124)
	FR	0.000 (0.000, 0.002)	—	0.001 (0.001, 0.002)	—	0.001 (0.000, 0.001)	—
	GB	0.001 (0.000, 0.003)	0.015 (0.010, 0.016)	0.005 (0.004, 0.006)	0.008 (0.004, 0.012)	0.003 (0.002, 0.004)	0.011 (0.009, 0.014)
	SA	0.394 (0.226, 0.551)	0.859 (0.858, 0.870)	0.360 (0.219, 0.515)	0.871 (0.869, 0.873)	0.378 (0.257, 0.498)	0.865 (0.863, 0.870)
	US	0.000 (0.000, 0.000)	—	0.092 (0.064, 0.128)	—	0.046 (0.032, 0.064)	—

[a] Two-letter country codes are shown as per ISO 3166-1 alpha-2 standard: AE = Arab Emirates; BD = Bangladesh; CA = Canada; EG = Egypt; FR = France; SA = Saudi Arabia; UK = United Kingdom; US = United States of America.

[b] Fractions of total probability density for distributions of Incubation period, age at exposure and their joint distribution, as per ‘Materials and methods’ section.

[c] Calculated over patient’s lifetime.

[d] Calculated over the portion of the patient’s lifetime falling between 1980 and 1996.