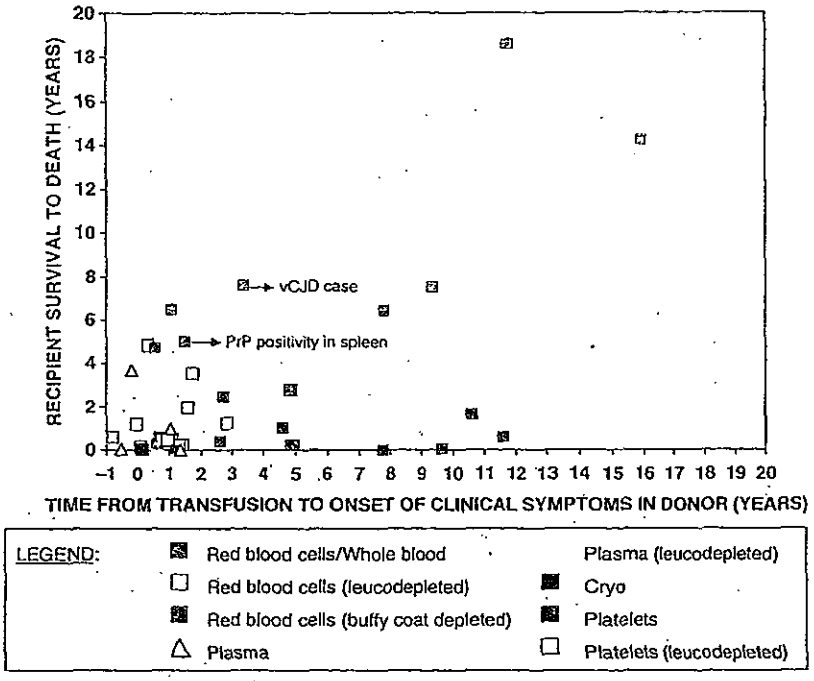

Fig. 1 Survival period (transfusion to death) for recipients of variant Creutzfeldt–Jakob disease components according to interval between transfusion and onset of clinical symptoms in the donor.

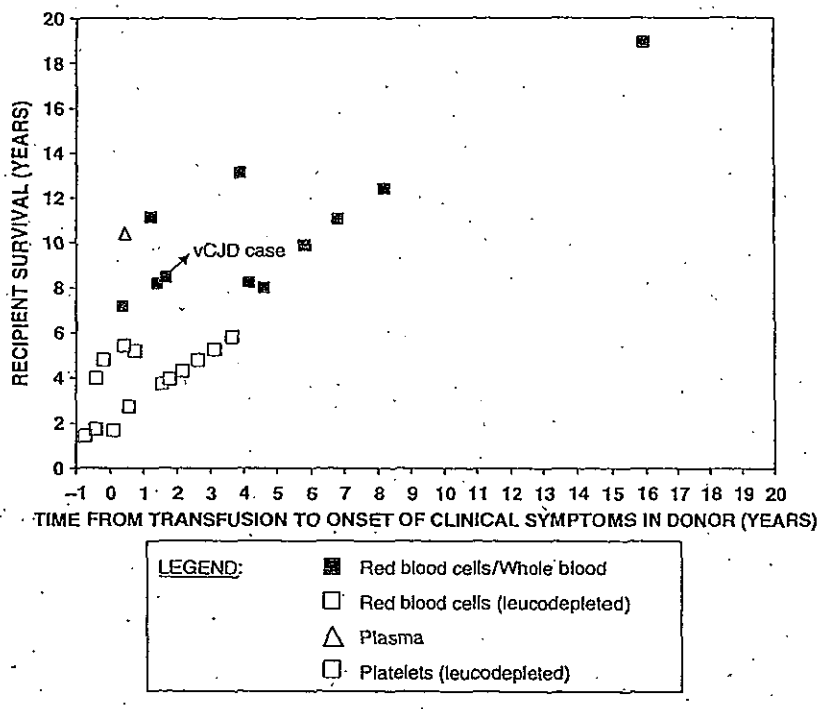

Fig. 2 Current survival period (since transfusion to 1 March 2006) for recipients of variant Creutzfeldt–Jakob disease components according to interval between transfusion and onset of clinical symptoms in the donor.

'dementia' recorded on the death certificate, but examination of case notes indicated that neither case had features to suggest vCJD. All the other recipients were certified as dying of causes unrelated to vCJD, except for a recipient whose cause of death on the death certificate was recorded as '1A dementia and II. prostate cancer' and was later confirmed neuropathologically as suffering from vCJD [7]. This patient, who had received a transfusion of red cells 6.5 years before onset of clinical symptoms, was a methionine homozygote at codon 129 of the human prion protein gene (*PRNP*). The case that donated to this individual also had a neuropathological diagnosis of vCJD, with clinical onset approximately 40 months after donating. In a second red cell recipient (of a different donor who developed clinical symptoms approximately

Table 2 Cause of death of variant Creutzfeldt–Jakob disease recipients known to have died (n = 40)

Interval from transfusion to death	Number of recipients	Cause of death
< 1 month	7	Acute renal cortical necrosis
		Cancer (2)
		Myocardial infarction
		Septicaemia (2)
		Sepsis (pancreatitis)
1– < 6 months	11	Aspiration pneumonia (sigmoid resection)
		Cancer (4)
		Myelodysplasia (2)
		Myelofibrosis
		Peritonitis (2)
		Stroke/diabetes mellitus/dementia
6– < 12 months	3	Cancer (3)
1– < 5 years	12	Acute myeloid leukaemia (2)
		Bronchopneumonia/senile dementia
		Cancer
		Ischaemic heart disease (3)
		Chronic obstructive airways disease (COAD)
		Hypertensive heart disease, chronic renal failure
		Myelodysplasia
		Disseminated sepsis
		Spinal haemangioblastoma
5– < 10 years	5	Cerebrovascular accident
		Ischaemic heart disease
		Acute lymphoblastic leukaemia
		Dementia[a], prostatic cancer
		Ruptured aortic abdominal aneurysm/severe atheroma/COAD[b]
≥ 10 years	2	Bronchopneumonia
		Ischaemic heart disease

[a]Confirmed variant Creutzfeldt–Jakob disease case [7].
[b]PrP positivity in lymphoid tissue, pre- or subclinical vCJD infection [8].

18 months after donating and was later diagnosed with neuropathologically confirmed vCJD), protease-resistant prion protein (PrP^{res}) was detected in the spleen and one lymph node (but not in the brain) at post-mortem [8]. This recipient, who died 5 years after transfusion without any clinical symptoms of vCJD, was a codon 129 *PRNP* heterozygote and is thought to represent pre- or subclinical infection.

Live recipients

Twenty-six recipients (39%) are alive as of 1 March 2006 with a mean age of 63 ± 19 years. Table 3 shows the number of live recipients according to the time elapsed since transfusion, along with their current age, component transfused and the interval between donation and onset of clinical symptoms of vCJD in the donor. Fifty per cent of live recipients were transfused with components from vCJD donors whose donations were made within 20 months of clinical onset, in seven cases around the time of development (n = 3) or shortly after (n = 4) the first signs of clinical illness. These cases would have appeared healthy when attending donor sessions and passed the normal medical checks as being fit to donate. Sixteen recipients have survived longer than 5 years, with six surviving > 10 years (one for over 18 years). These patients, mean age currently 61 ± 19 years, were given blood from donors who developed vCJD symptoms at intervals ranging from around 5 months to 191 months after making the donation (see Table 3). Recently, a diagnosis of probable vCJD has been made in one of these surviving recipients who had received a transfusion of red cells 7 years and 10 months before onset of clinical symptoms [9]. The donor of this third probable transfusion-transmitted vCJD infection developed vCJD approximately 21 months after the donation, and the recipient is a codon 129 *PRNP* methonine homozygote.

Plasma for UK fractionation

Twenty-five units of plasma originating from 11 different donors, bled between 6 months and 17 years, 11 months before onset of clinical vCJD symptoms, were supplied for UK fractionation during the period 1986–1998. Product batches manufactured from 23 plasma units derived from nine donors have been traced. The fate of batches of product derived from the two remaining plasma donations, from two different donors, has not yet been traced, and this search is still ongoing. Table 4 lists the plasma products derived from the 23 traced donations and the number of batches implicated, divided into risk categories as used in the plasma product notification exercise (www.hpa.org.uk/infections/topics_az/cjd/Recommendations.pdf). The fate of batches of products has not been traced to individual recipients as part of this study. It is known, however, that haemophilia centres have traced the ultimate fate of the batches of factor VIII. It is also known that no case of vCJD has been identified in a patient with haemophilia in the UK.

sCJD cases with history of blood donation

Ninety-three cases of sCJD identified between 1980 and 2000 were reported to have been blood donors, with only 38 reported to have donated from 1980 onwards. Donation records for most sCJD cases were untraceable since most dated back many years before 1980, in some cases to the 1940s. Donation records were found for eight sCJD cases; but only three had actually donated labile blood components for hospital use (one with 18 recipients, and one each with one recipient) which could be traced to recipients. A total of 20 recipients were transfused between 1995 and 1999 with components from these three donors who went on to develop

Table 3 Live recipients of labile blood components donated by variant Creutzfeldt–Jakob disease cases (n = 26)

Time elapsed since transfusion[a]	Current age of recipient (years)[a]	Blood component transfused	Interval between blood donation and onset of clinical symptoms in donor (months)[b]
1 – < 2 years	48	Platelets (leucodepleted)	–9 months
	49	Red cells (leucodepleted)	–5 months
	83	Red cells (leucodepleted)	2 months
2 – < 3 years	38	Red cells (leucodepleted)	7 months
3 – < 4 years	58	Red cells (leucodepleted)	19 months
	83	Red cells (leucodepleted)	22 months
	90	Red cells (leucodepleted)	–5 months
4 – < 5 years	59	Red cells (leucodepleted)	26 months
	67	Red cells (leucodepleted)	–2 months
	89	Red cells (leucodepleted)	32 months
5 – < 6 years	30	Red cells (leucodepleted)	37 months
	52	Red cells (leucodepleted)	9 months
	64	Red cells (leucodepleted)	44 months
	71	Red cells (leucodepleted)	5 months
6 – < 7 years	–	–	–
7 – < 8 years	42	Red cells	5 months
8 – < 9 years	31[c]	Red cells	21 months
	74	Red cells	17 months
	76	Red cells	49 months
	87	Red cells	55 months
9 – < 10 years	75	Red cells	70 months
> 10 years	33	Cryo-depleted plasma	7 months
	49	Red cells	15 months
	67	Red cells	46 months
	70	Red cells	191 months
	75	Red cells	98 months
	87	Red cells	82 months

[a]As at 1 March 2006.

[b]A negative interval denotes that donation was made by individual while (retrospectively recognized) clinical symptoms were present.

[c]Probable variant Creutzfeldt–Jakob disease case [9].

sCJD between 1 and 5 years after donation. Of these, 11 (55%) received red cell components, eight recipients (40%) received platelets and one (5%) received fresh frozen plasma.

As of 1 March 2006, 12 recipients are confirmed dead with a mean age at death of 74 ± 15 years. Of these, five died soon after transfusion (four within a week, and one 2 months later) and seven survived for between 1 and 8 years after receiving their transfusion before dying of a variety of non-CJD-related causes (cerebrovascular accident/stroke, n = 3; acute myeloid leukaemia, n = 3, general debility/old age, n = 1). Seven recipients are not known to be dead from ONS flagging to date, and are therefore presumed to be alive. The mean age of these seven recipients is 58 ± 19 years. The time elapsed since their transfusion ranges from 7 to 9 years. The fate of a further recipient is unknown. None of the sCJD recipients identified as having received blood from donors who went on to develop sCJD have appeared on the NCJDSU register to date.

fCJD cases with history of blood donation

Donation records were found for three out of five cases of fCJD identified between 1992 and 2000, all reported to have donated blood after 1980. These three cases had all donated labile blood components (one with five recipients, one with four recipients and one with two recipients) for hospital use which could be traced to individual recipients. A total of 11 recipients were transfused between 1977 and 1992 with labile components from these three donors who went on to develop fCJD between 1 and 15 years later. Nine of the 11 (82%) recipients received red cell components (whole blood, n = 6, red cells n = 3) while two received platelets.

Five of 11 recipients identified have since died with a mean age at death of 75 ± 6 years. Three of these survived for 3, 10 and 17 years after transfusion before dying of non-CJD-related causes (cancer, n = 2; bronchopneumonia, n = 1); and two died of cancer shortly after receiving their transfusion.

Table 4 Product batches made by UK fractionators derived from plasma donated by individuals who later developed variant Creutzfeldt–Jakob disease[a,b]

Infectivity Classification[c]	Plasma product	Number of implicated batches
Low	Factor VIII (excipient[d])	77
	Albumin 20%	21
	i.m. immunoglobulin	12
Medium	*Albumin 4·5%*	28
	i.v. immunoglobulin	11
High	Factor VIII	16
	Factor IX	8
	Anti-thrombin	1
	TOTAL	174

[a]Twenty-three plasma donations from nine variant Creutzfeldt–Jakob disease donors, data courtesy of Health Protection Agency.
[b]Excludes fate of two plasma units from two further vCJD cases (see text for explanation).
[c]Risk categories as used in plasma product notification exercise.
[d]Albumin from implicated plasma donation used as excipient (inert substance added to provide bulk) in preparation of batch of Factor VIII.

Three recipients are not known to be dead from ONS flagging to date, and are therefore presumed to be alive. The mean age of these three recipients is 44 ± 20 years. The time elapsed since their transfusion ranges from 13 to 21 years. The fate of a further three recipients is not known. None of the fCJD recipients identified as having received blood from donors who went on to develop fCJD have appeared on the NCJDSU register to date.

vCJD cases with history of transfusion

Eleven vCJD cases were reported to have received past blood transfusions between 1962 and 1999. A further case received a blood transfusion after onset of illness. This case is excluded from further analysis. For two cases, hospital records showed that they had not been transfused. No hospital records could be found for another two cases reported to have been transfused in 1962 and 1971, respectively. Hospital transfusion records were found for seven vCJD cases (64% of those reported as transfused) who had been transfused with components donated by 125 donors (121 identified), with one vCJD case, who also received a solid organ transplant, receiving components from 103 donors. The identity of four donors who donated red cell/whole blood components to two cases (case 2 and case 7, see Table 5) is unknown. Table 5 shows the transfusion date, number of donors and blood components donated, and the interval from transfusion to onset of clinical symptoms of vCJD in these seven recipients. These cases had been exposed to between two and 103 donors, respectively (NB search for donors to case 6 is incomplete). To date, one donor who gave red cells to case 5 and another donor who gave red cells to case 6 are also registered on the NCJDSU database as vCJD cases. These are the donors of the two clinical cases of transfusion-transmitted vCJD referred to previously (see vCJD cases with history of donation).

sCJD cases with history of transfusion

Fifty-two cases of sCJD identified between 1980 and 2000 were reported to have received a blood transfusion, of which 28 received a transfusion after 1980. Transfusion records were found for seven sCJD cases transfused between 1984 and 1997. Donor details were found for 24 donors who donated components transfused to these seven sCJD cases. One of these donors is known to have died, with a cause of death not related to CJD. Twenty donors are not known to have died from ONS flagging to date, and are therefore presumed to be alive. The fate of a further three donors is not known. The mean age of the donors presumed still alive is 51 ± 9 years. None of the traced donors who gave blood to patients who were subsequently diagnosed with sCJD have appeared on the NCJDSU register to date.

fCJD cases with history of transfusion

One case of fCJD identified in 1992 was reported to have received three blood transfusions in 1965, 1970, and 1987 none of which could be traced.

Discussion

This study has identified three instances in which a recipient of a transfusion derived from a 'vCJD' donor has developed infection with vCJD, including two clinical cases and one pre- or subclinical infection [7–9]. These are three different donor/recipient pairs. In view of the small size of the total at-risk recipient population ($n = 66$) and the background mortality rate for vCJD in the general UK population (0·24/million/annum), these observations provide strong evidence that vCJD can be transmitted from person to person through blood transfusion. This finding has had important implications for public health policy nationally and internationally.

The risk of developing vCJD infection in the surviving recipient population is significant but cannot be precisely estimated because of variables including the timing of blood donation in relation to clinical onset in the donor, the influence of the codon 129 genotype of donor and recipient and the effect of the introduction of leucodepletion in 1999. Furthermore, the currently observed number of infections in the recipient population may be an underestimate as some surviving recipients may yet develop vCJD and there is limited available information on the outcome in the cohort of

Table 5 Donors (n = 125) of labile blood components given to variant Creutzfeldt–Jakob disease cases[a] (n = 7) with identifiable past hospital transfusion records

Case	Transfusion date	Number of donors of labile blood components transfused	Blood component donated to vCJD recipient	Interval from transfusion to onset of illness
1	1993	38	Cryoprecipitate (4) Fresh frozen plasma (11) Platelets (8) Red cells (14) Whole blood (1)	4 years, 9 months
1	1993	65	Cryoprecipitate (12) Fresh frozen plasma (25) Platelets (17) Red cells (11)	4 years, 6 months
2	1983	2[b]	Red cells	15 years, 11 months
2	1993	3	Fresh frozen plasma	6 years, 3 months
3	1994	4	Red blood cells	5 years, 4 months
4[c]	1999	5	Red blood cells (2) Red blood cells (Leucocyte-depleted) (3)	8 months
5[a]	1996	5[d]	Red blood cells	6 years, 6 months
6[a]	1997	1+[e]	Red blood cells	7 years, 10 months
7	1982	2[b]	Whole blood	13 years, 11 months

[a]Two of these cases linked to donors already on the National CJD Surveillance Unit (NCJDSU) register as vCJD cases [7,9].
[b]Component details traced, but donors not identifiable.
[c]Timing of clinical illness excludes blood transfusion as the source of infection in this case.
[d]One of the donors already on NCJDSU register as vCJD case, others presumed not to be source of infection.
[e]One donor already on NCJDSU register as vCJD case. Search for 40+ donors to Case 6 not complete, as of 1 March 2006.

deceased recipients; a significant proportion of these individuals may not have survived long enough to express clinical disease even if infected. The minimum incubation period in CJD transmitted from person to person by a peripheral route is 4·5 years in kuru and growth-hormone-related CJD [10,11] and only nine deceased recipients survived for longer than this period. An investigation of the hospital records of the deceased recipients is underway, and to date, none had clinical features of vCJD pre-mortem. However, the identification of the individual with 'preclinical' vCJD infection was dependent on post-mortem examination of peripheral lymphoreticular tissues, and, to date, no equivalent tissues have been available in the deceased transfusion recipients. Extrapolating from the three observed infections in the total recipient population is likely to lead to an underestimate of the overall risk of transfusion transmission of vCJD, although the introduction of leucodepletion in 1999 may have reduced the risk to recipients transfused after this date.

A further important variable in estimating individual risk is the time from blood donation to clinical onset in the donor and, although evidence from animal studies in relation to this issue is conflicting [12–14], it is likely that an extended gap between blood donation and clinical onset in the donor will reduce the risk of transfusion transmission. All tested clinical cases of vCJD have been methionine homozygotes at codon 129 of *PRNP*, but the individual with 'pre-clinical' transfusion transmitted infection was heterozygous at this locus [8], indicating that individuals with this genotype are susceptible to secondary infection with vCJD. Except for the three cases infected through blood transfusion, the codon 129 genotypes of the recipient population are not known. Although the relative risk of secondary infection in relation to the codon 129 genotype is uncertain, a recent study in a transgenic mouse model suggests that individuals with all human codon 129 genotypes may be susceptible to secondary infection with vCJD, with a hierarchy of risk from methionine homozygotes to heterozygotes to valine homozygotes [15]. Risk may vary according to genetic background, but it cannot be assumed that some recipients will possess an absolute genetic barrier to infection.

The analysis of vCJD cases with a history of blood transfusion has identified over 100 donors to these cases, although the great majority were linked to one vCJD case who had undergone an organ transplant. A risk assessment has suggested that these donors are themselves at significant risk of developing vCJD and these individuals have been informed of this risk and have been advized not to act as blood or organ donors. To date, none of these individuals have developed vCJD, with the exception of the two donors linked to the two clinical cases of vCJD described above.

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Effectiveness of leucoreduction for removal of infectivity of transmissible spongiform encephalopathies from blood

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In 1999, the UK implemented universal leucoreduction as a precaution against transmission of variant Creutzfeldt-Jakob disease by transfusion of domestic blood or red blood cells. We aimed to assess how effectively leucoreduction reduced infectivity of transmissible spongiform encephalopathies (TSEs) in blood. 450 mL of whole blood collected and pooled from scrapie-infected hamsters was leucoreduced with a commercial filter. Blood cell concentrations were quantified, and infectivity titres measured. Blood cell recovery and white blood cell removal complied with American Association of Blood Banks standards. Leucofiltration removed 42% (SD 12) of the total TSE infectivity in endogenously infected blood. Leucoreduction is necessary for the removal of white-cell-associated TSE infectivity from blood; however, it is not, by itself, sufficient to remove all blood-borne TSE infectivity.

Transmissible spongiform encephalopathies (TSEs) are fatal CNS infections that can incubate asymptomatically for a decade or more in human beings before the appearance of clinical disease. People in the asymptomatic phase of variant Creutzfeldt-Jakob disease (vCJD) appear healthy and donate blood with the same frequency as any healthy person. Transmission of vCJD by transfusion was recently recognised in Great Britain.[1] To reduce the risk of transfusion transmission of such diseases in human beings, the UK implemented universal leucoreduction of donated blood in 1999. This measure was based on the expectation that infectivity would be associated with white blood cells.[2] However, findings in blood from infected mice and hamsters suggested otherwise; at least 40% of the infectivity was plasma-associated, suggesting that leucoreduction would not eliminate infectivity (Rohwer laboratory, unpublished).[3] Other investigations showed no loss of infectivity when small amounts of TSE-infected plasma were passed through scaled-down filters.[4] Similarly, no significant removal of abnormal prion protein was detected when units of human whole blood, spiked with a microsomal fraction from TSE-infected brain, were passed through leucoreduction filters from any of the four major suppliers.[5] Because of reservations about the relevance of these experiments, none of these findings aroused concern.

We investigated the effectiveness of leucoreduction in removal of TSE infectivity from a human-sized unit of pooled hamster blood. To ensure that the 150 hamsters needed for a 450 mL blood pool were at the same symptomatic stage of disease (wobbling gait and head bobbing) for each of two separate experiments, 400 weanling golden Syrian hamsters (Harlan, Madison, WI, USA) were inoculated intracranially with 50 µL of brain homogenate containing about 250 infectious dose$_{50}$ (ID$_{50}$) of hamster-adapted scrapie-strain 263K. A low dose of infectivity was used to preclude re-isolation of the inoculum in the blood. This animal protocol was approved by the University of Maryland Institutional Animal Care and Use Committee.

We obtained two pools of blood from the hamsters, one at 106 days and one at 111 days after inoculation. Under carbon dioxide anaesthesia, 3·5 mL of blood was drawn from the right ventricle into 0·5 mL of CP2D anticoagulant. Care was taken not to touch any other tissue. Only perfect bleeds containing 12·5% CP2D with no visible clots were pooled.

Two in-line leucofiltration systems from Pall Corporation (Port Washington, NY, USA) were evaluated. We selected the Leukotrap WB collection set for the infectivity study because filtration and component separation of hamster blood was fully compliant with American Association of Blood Banks (AABB)[6] specifications, and required only two titrations for interpretation. The Leukotrap RC-PL system

	Volume (mL)*	White blood cells†		Red blood cells, total (% of total)	Platelets, total (% of total)
		Total (% of total)	Log$_{10}$ reduction		
Whole blood	448·5	$2·1\times10^{9}$ (100%)	0	$3·7\times10^{13}$ (100%)	$1·4\times10^{12}$ (100%)
Leucoreduced blood	424·2	$3·0\times10^{6}$ (0·15%)	2·9	$3·6\times10^{13}$ (100%)	$1·5\times10^{12}$ (100%)
Plasma	179	$3·0\times10^{5}$ (0·02%)	3·8	0 (0%)	$1·1\times10^{12}$ (8%)
Red blood cells + AS3	305·9	$2·0\times10^{6}$ (0·15%)	3	$3·1\times10^{13}$ (86%)	1×10^{11} (71%)

*Volume measurements were obtained by weight using experimentally determined densities of whole hamster blood, 1·04 g/mL. †Values are average of at least three separate microscopic determinations using a haemocytometer and by flow cytometric measurements with white cells stained with propidium iodide. AS3 is a preservative and stabiliser.

Table 1: Blood component cell numbers and volumes before and after leucoreduction