Prion infectivity is closely related to neurological disorders called TSE which include human diseases such as Creutzfeldt–Jakob disease (CJD), Fatal Familial Insomnia and Gerstmann–Straussler Scheinker syndrome.

CJD is classified as sporadic, genetic, iatrogenic including the CJD variant associated with bovine spongiform encephalopathy. The infectious agent responsible for this disease was called a "prion" by Prusiner [1]. It is a glycoprotein which is normally present in the physiological form (PrP^c) which becomes pathological in CJD (PrP^{sc}). The transition process from the physiological to the pathological form is complex [2]. Scientists have demonstrated a trans-conformational change between both protein forms [3]. PrP^c primarily contains α helix, though PrP^{sc} contains more β sheets in its three-dimensional structure [4]. CJD is characterized by intra-cerebral accumulation of abnormal prion protein which is partially protease resistant (PrP^{res}). Cleavage of PrP^{sc} by proteinase K results in two types of PrP^{res} after western blot, type 1 and type 2, according to Parchi's classification [5].

Studies on the elimination of viral contamination from biopharmaceutical products (which are prepared from human cells) have been conducted using nanofiltration [6–8]. This nanofiltration method has been effective on many viruses. Albumin solution safety nanofiltration has been conducted on scrapie prion protein [9].

The purpose of this research is to study the efficacy of nanofiltration on CJD PrP^{sc} in order to introduce this method into the manufacturing process of a therapeutic immunoglobulin solution Lymphoglobuline®.

Lymphoglobuline® equine anti-human thymocyte immunoglobulin is a selective immunosuppressive agent acting mainly on human T lymphocytes. It recognizes most of the molecules involved in the cascade of T-cell activation during graft rejection, such as T-cell receptor and CD3, HLA class 1 molecules, CD4 and CD8 co-receptors, co-activation molecules or adhesion molecules CD2, CD5, and CD18. The therapeutic indications are the followings:

- immunosuppression for transplantation: prevention and treatment of graft rejection;
- treatment of aplastic anemia.

During the process of purification of the equine anti-human thymocytes a step of nanofiltration was added for the viral security.

2. Materials and methods

2.1. Biopharmaceutical product

Lymphoglobuline® is an anti-thymocyte equine immunoglobulin that induces immunosuppression as a result of T-cell depletion and immune modulation. It is approved for the prevention and treatment of rejection episodes in kidney, pancreas or liver transplantation. In hematology, Lymphoglobuline® is approved for treatment of aplastic anemia and in the treatment of steroid resistant graft versus host disease.

In the Lymphoglobuline® manufacturing process, human thymocytes, membrane red blood cells and placenta are used. These human elements represent a virtual potential source of contamination of Lymphoglobuline®.

2.2. Human source of pathological prion protein

After the histological, immunohistochemical and biochemical analyses of post-mortem human brains, one case of definite CJD, and one non-CJD were chosen. The anatomic site chosen was the frontal cortex. The CJD case selected was characterized by the presence of PrP^{res} type 1 in western blot analysis according to Parchi's classification and by synaptic deposits of PrP^{sc} with an immunohistochemical technique. The same human cortex was used as source of PrP^{sc} for the reference scale and for the nanofiltration samples.

2.3. Sample preparation

2.3.1. Human brain homogenate

Frontal cortex of CJD and non-CJD cases was spiked in PBS buffer, 1:10 at final dilution. These homogenates were filtered successively with needles of 0.6 mm and 0.5 mm diameter in order to obtain homogenous preparation. After centrifugation at 1000g for 5 min, supernatants were applied to nanofiltration process.

2.3.2. Reference scale

This reference scale was prepared with series of dilutions of CJD brain homogenate in Lymphoglobuline® from 1:10 to 1:20,000. This reference scale was based on the technique used by Lee et al. [10,11].

2.3.3. Nanofiltration samples

These samples were prepared using CJD brain homogenate dilutions in Lymphoglobuline®. Three different samples were produced; samples at a high PrP^{sc} dilution (1:500), samples at a moderate PrP^{sc} dilution (1:100) and samples at a low PrP^{sc} dilution (1:10). Each dilution was prepared for three samples, one non-nanofiltrated (control) and two nanofiltrated. These samples were prepared as a reference scale with an adaptation of the method used by Lee et al. [11].

2.4. Filtration

Small-sized (membrane diameter: 47 mm) Pall® filters (hydrophilic Polyvinylidene fluoride microporous membrane) with mean pore sizes for Pall® DVD of about 0.1 μm, Pall® DV50 of about 50 nm and Pall® DV20 of about 20 nm were used successively in the nanofiltration process. The filtration mode was conducted at a constant membrane pressure of 3 bars. The samples underwent nanofiltration in the following order: negative control, CJD samples at a high PrP^{sc} dilution (1:500), CJD samples at a moderate PrP^{sc} dilution (1:100), CJD samples at a low PrP^{sc} dilution (1:10) and negative control (Fig. 1).

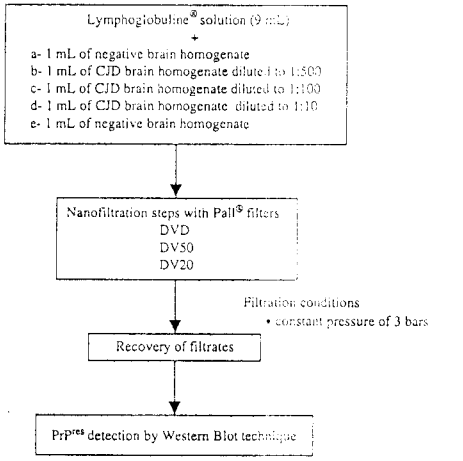

Fig. 1. Nanofiltration process. (a) Negative control sample at 1:10 in Lymphoglobuline®; (b) CJD sample at a high PrP^{sc} dilution (1:500) in Lymphoglobuline®; (c) CJD sample at a moderate PrP^{sc} dilution (1:100) in Lymphoglobuline®; (d) CJD sample at a low PrP^{sc} dilution (1:10) in Lymphoglobuline®; and (e) negative control sample at 1:10 in Lymphoglobuline®, ×2, produced in duplicate.

The nanofiltration material was treated with sodium hydroxide (2 M) for 1 h between each nanofiltration of different PrP^{sc} dilution samples.

2.5. PrP^{res} detection

The western blot technique was used to detect PrP^{res} after proteinase K treatment [12]. The anti-prion protein antibody revealed three strips of a molecular weight between 30 and 22 kDa (Fig. 2) corresponding to the biglycosylated, monoglycosylated and unglycosylated forms. Then, PrP^{res} was revealed by chemiluminescence. This technique was used to detect PrP^{res} in reference scale samples and in samples before and after nanofiltration.

The reference scale samples and samples for nanofiltration were produced and developed by the western blot technique under the same conditions and in the same time.

2.6. Determination of reduction factors

The reduction factors defined as the reduced titer versus the real titer present in the spiked sample were determined by comparing the PrP^{res} signal of samples before and after nanofiltration with the PrP^{res} signal of reference scale. After this comparison, we determined a reduction factor (log) for each sample.

3. Results

The reference scale ranges from 1:10 to 1:20,000 dilutions of CJD brain homogenates. From the 1:10 to 1:2000 dilutions,

processes [14]. Effective methods include for example exposure to 1 M sodium hydroxide during autoclaving at 121 °C. This kind of method using chemical agents (sodium hydroxide, chlorine at high concentrations) and physical treatment by autoclaving is very drastic and it is a real problem to inactivate PrP^{sc} in biopharmaceutical products without modifying their therapeutic properties. The reduction of any risk associated with a pharmaceutical product will be dependent on the physical removal of infective material during product manufacture. Many techniques for plasma-derived products, such as ethanol fractionation, depth filtration and chromatographic processes, may contribute to a significant partitioning of prion protein [10,15–18]. Although early applications of nanofiltration targeted viral removal [7,8,19], new data suggest that it may be a specific removal system for prion proteins as well. Human TSE pathogens in diluted brain homogenate were reported to be removed by a Millipore screen-type 0.025 μm membrane filter employed during production of growth hormone [20]. However, only a small quantity of diluted brain homogenate could pass through the membrane. Planova® cartridges with mean pore sizes from 75 to 10 nm were used to filter brain homogenate from mice infected with human TSE [21]. No infectivity was detected in the 35 nm filtrate. The pathogenic agent was estimated to be approximately 40 nm in size. However, some residual infectivity was found in the 10 nm filtrate when 1% Sarkosyl was added to the homogenate [22]. Recently, removal of scrapie agent ME7, a mouse adapted strain of scrapie used as a model for the BSE or vCJD agents by using nanofiltration of a 2% albumin solution spiked with a brain homogenate [23]. The albumin recovery was over 90%. Extent of removal was influenced by the filter type and by the addition of an anionic detergent (Sarkosyl) to the protein solution. An infectivity of 4.93 and 1.61 log was removed using a 35-nm filter without and with detergent, respectively. Moreover, a reduction of infectivity of >5.87 and 4.21 log was obtained using a 15-nm filter in the absence and presence of detergent, respectively. No residual infectivity was detected in any filtrate when using 15 nm or smaller porosity filters. Studies have shown an efficacy of 35–15 nm filters in achieving some removal of prions from biological solutions with the best removal with a 15-nm filter. The data, although encouraging, should be analyzed more accurately due to the tendency of prion spikes to aggregate under the experimental conditions used and with human prion protein because this removal could be dependent on the "strain" of prion protein.

In our study, we wanted to study the efficacy of nanofiltration on human PrP^{sc} in a biopharmaceutical product (Lymphoglobuline®). We used human PrP^{sc} from CJD patients as the contaminant. This contamination condition was important to study the Lymphoglobuline® nanofiltration technique under conditions as close as possible to a possible contamination by human cells used for the preparation of this product. The extent of removal may be influenced by the aggregation, type [24] and conformation of prion proteins and the physicochemical nature of the solution filtered. These parameters were important to choose the PrP^{res} type for the study. Amyloid plaques or focal deposits of PrP^{sc} still remain after homogenizing the cerebral cortex and the hypothesis was made that this kind of PrP^{sc} aggregation could be the result of a bias in the methodology. For this reason, PrP^{res} type 1 associated with synaptic deposits with an immunohistochemical technique was chosen in order to test the Lymphoglobuline® nanofiltration process under worst conditions to test the filters. In this study, Lymphoglobuline® was spiked with brain homogenate at different dilutions (1:10, 1:100, and 1:500). These PrP^{sc} dilutions can be correlated with World Health Organization (WHO) classification of organ infectivity: the low PrP^{sc} dilution corresponding to 1:10 (brain and spinal cord), moderate PrP^{sc} dilution corresponding to 1:100 (spleen, tonsil, lymph node, intestine, placenta…) and high PrP^{sc} dilution corresponding to 1:500 (brain stem, thymus, liver, pancreas, lungs…).

The comparison of the samples before and after nanofiltration showed a reduction factor between 3.3 and 1.6 log in comparison with the reference scale. The reduction factor of samples at a low PrP^{sc} dilution (1:10) was between 3 and 3.3 log. This dilution could correspond to a brain or a spinal cord PrP^{sc} concentration (WHO). The reduction factors for a very high PrP^{sc} concentration obtained illustrate a very good efficacy of the nanofiltration process.

In samples at a moderate PrP^{sc} dilution (1:100) and samples at a high PrP^{sc} dilution (1:500), the PrP^{res} strips were not detected after nanofiltration, the reduction factor was strictly greater than 2.3 and 1.6 log, respectively. The 1:100 dilution could correspond at a spleen or tonsil or lymph node or intestine or placenta PrP^{sc} concentration (WHO) and the 1:500 dilution could correspond to a brain stem or thymus or liver or pancreas or lungs PrP^{sc} concentration (WHO). In conclusion, the data obtained on both these PrP^{sc} dilutions are encouraging because, after nanofiltration, the PrP^{res} signal was not detected, although they are only indicative with probably underestimated reduction factors. Finally, the reduction factor obtained is 3.3 log and seem to demonstrate the efficacy of the nanofiltration process on human CJD PrP^{sc} with a good protein recovery.

Removal may be based on a sieving mechanism or due to adsorption on the membrane. The potential to use nanofiltration as a dedicated step for prion removal may have a significant impact on the safety of biopharmaceutical products and recombinant proteins, when production involves the use of human or animal derived materials, or medicinal products derived from bovine sources [25,26]. This technique has the ability to extend the concept of sterility of biological products from bacteria to, at least, some viruses. Our results suggest that nanofiltration could be also of interest for the removal of human pathological prion proteins.

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ORIGINAL PAPER

Creutzfeldt–Jakob disease and blood transfusion: results of the UK Transfusion Medicine Epidemiological Review study

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Background and Objectives This paper reports the results to 1 March 2006 of an ongoing UK study, the Transfusion Medicine Epidemiological Review (TMER), by the National CJD Surveillance Unit (NCJDSU) and the UK Blood Services (UKBS) to determine whether there is any evidence that Creutzfeldt–Jakob disease (CJD), including sporadic CJD (sCJD), familial CJD (fCJD), and variant CJD (vCJD) is transmissible via blood transfusion.

Materials and Methods Sporadic CJD and fCJD cases with a history of blood donation or transfusion are notified to UKBS. All vCJD cases aged > 17 years are notified to UKBS on diagnosis. A search for donation records is instigated and the fate of all donations is identified by lookback. For cases with a history of blood transfusion, hospital and UKBS records are searched to identify blood donors. Details of identified recipients and donors are checked against the NCJDSU register to establish if there are any matches.

Results CJD cases with donation history: 18/31 vCJD, 3/93 sCJD, and 3/5 fCJD cases reported as blood donors were confirmed to have donated labile components transfused to 66, 20, and 11 recipients respectively. Two vCJD recipients have appeared on the NCJDSU register as confirmed and probable vCJD cases. The latter developed symptoms of vCJD 5.5 years and 7.8 years respectively after receiving non-leucodepleted red blood cells (RBCs) from two different donors who developed clinical symptoms approximately 40 and 21 months after donating. A third recipient, given RBC donated by a further vCJD case approximately 18 months before onset of clinical symptoms, had abnormal prion protein in lymphoid tissue at post-mortem (5-years post-transfusion) but had no clinical symptoms of vCJD. CJD cases with history of transfusion: Hospital records for 7/11 vCJD and 7/52 sCJD cases included a history of transfusion of labile blood components donated by 125 and 24 donors respectively. Two recipients who developed vCJD were linked to donors who had already appeared on the NCJDSU register as vCJD cases (see above). No further links were established.

Conclusion This study has identified three instances of probable transfusion transmission of vCJD infection, including two confirmed clinical cases and one pre- or post-clinical infection. This study has not provided evidence, to date, of transmission of sCJD or fCJD by blood transfusion, but data on these forms of diseases are limited.

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Introduction

Until 2004, it was generally accepted that Creutzfeldt–Jakob disease (CJD) had not been transmitted by blood transfusion.

Preliminary findings from sheep studies indicate that bovine spongiform encephalopathy (BSE) and scrapie can be transmitted by blood transfusion [1,2]. It is vital to find out whether this also applies to human transmissible spongiform encephalopathies (TSEs) and, in particular, variant CJD (vCJD). The UK is the only country where a significant outbreak of vCJD has occurred and is in a unique position to study this question which has important implications for public health policy. The results reported in this paper are from a study which is being carried out, with ethical approval, to investigate whether or not there is any evidence for the transmission of any type of CJD (sporadic, familial and variant) by blood transfusion.

Materials and methods

CJD surveillance

A surveillance system for CJD, the National CJD Surveillance Unit (NCJDSU), was established in the UK in 1990 with the aim of identifying all cases of CJD in the UK. The methodology of this study has been described previously [3]. In brief involves referral of suspected cases to the Unit by targeted professional groups, including neurologists, neuropathologists, review of suspects by a neurologist in the Unit and review of investigation results and neuropathological material when available. Cases are classified according to standard diagnostic criteria [4,5]. Onset of clinical symptoms for vCJD cases are estimated to the nearest month by NCJDSU on the basis of available clinical information. Details of past medical history, including blood donation or transfusion, are obtained from the family of suspected cases. Following the identification of vCJD in 1996 a collaborative study, the Transfusion Medicine Epidemiological Review (TMER), was established between the NCJDSU and UK Blood Services (UKBS) to search for evidence of transfusion transmission of CJD. The study was granted ethical approval by the local Research Ethics Committee.

Notification of CJD cases with a history of donation

Sporadic CJD (sCJD) and familial CJD (fCJD) cases with a history of blood donation are notified to UKBS retrospectively. For vCJD, all patients who are old enough to have donated blood (> 17 years of age) are notified to UKBS at diagnosis whether or not there is a known history of blood donation. Upon receipt of notification from the NCJDSU, a search is made for donor records. Current computer databases and archived records (computerized and paper-based records where appropriate) based at individual blood centres are searched using name, date of birth, and previous address as identifiers. For CJD cases reported as blood donors, information on dates and places of donation is also used to ide

Table 1 Recipients of blood donated by variant Creutzfeldt–Jakob disease cases by year and blood component transfused ($n = 66$)

Year of transfusion	Blood component transfused	Number of recipients
1980–1984	Whole blood	1
	Red blood cells	1
1985–1989	Red blood cells	2
1990–1994	Red blood cells	9
1995–1999	Whole blood	1
	Red blood cells	15
	Red blood cells – buffy coat depleted[a]	2
	Red blood cells – leucodepleted[b]	2
	Fresh frozen plasma	3
	Cryo-depleted plasma	1
	Cryoprecipitate	1
	Platelets (pooled)	1
2000–2004	Red blood cells – leucodepleted	23
	Fresh frozen plasma – leucodepleted	2
	Platelets (pooled, leucodepleted)	2

[a]Red cells with buffy-coat (containing most of the platelets and white cells) removed by centrifugation and physical separation.
[b]Red cells leucocyte-depleted by pre-storage filtration to $< 5 \times 10^6$/unit according to UK guidelines [6].

2006. Of these, 31 of 150 (21%) were reported to have been blood donors at various times in the past, although there is variation in the details of available information and the confidence of families in donation history.

Donor records were found for 24 vCJD cases, comprising 20 reported by relatives as blood donors and four additional cases with no reported donation history. Of these, 18 vCJD cases (12% of the total eligible to donate blood) were confirmed to have donated labile blood components, with the number of components made and issued for use in UK hospitals ranging from 1 to 14 per donor. Six vCJD cases were registered as donors, but had not donated labile blood components. Two of these had never attended sessions, three were deferred (due to past medical history, low haemoglobin value and illness, respectively) and one case had donated plasma for fractionation only (made from a single donation from which the red cells were discarded).

The search for donor records was negative in 11 of 31 (35%) vCJD cases reported as putative donors (three of whom allegedly donated well before the onset of the BSE epidemic in the 1980s). The information provided in these negative cases was minimal, except in one case where relatives were confident that regular donations (up to 50) had been made in the years leading up to 1993. Despite extensive searches no records were found; moreover, blood collection sessions had never been made at the purported venue. No explanation has been found for the lack of records, although discrepancies in some of the details given suggest that the history was not as certain as initially thought.

Labile components issued to hospitals

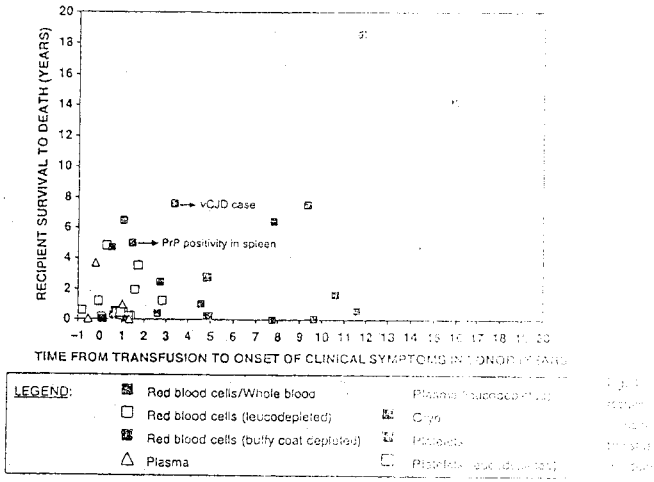
Sixty-six labile components originating from 18 donors were issued to UK hospitals over the period 1981–2004 and transfused to patients according to blood transfusion laboratory records. A further nine components issued between 1982 and 1996 could not be traced by the relevant hospital. Table 1 gives the number of recipients transfused by year and the type of blood component transfused. Fifty-six recipients (85%) received red cells or whole blood, seven (11%) were transfused with labile plasma components or derivatives and three (4%) received pooled platelets made according to UK specifications in which the buffy-coat preparation containing platelets from the implicated vCJD donor was pooled with buffy coats from three other donors and resuspended in plasma from one of the four donations. Nearly half of the red cell recipients received red cells that had been leucocyte-depleted by pre-storage filtration to $< 5 \times 10^6$ leucocytes per unit (in 99% of units with 95% statistical confidence according to UK guidelines [6]) after the introduction of universal leucocyte depletion of the UK blood supply in 1999.

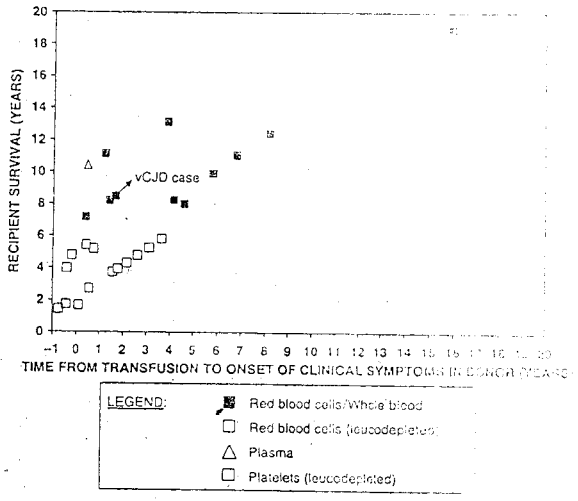
Recipients of blood components

Patient identifiers are available for 66 recipients who received blood from 18 different donors who went on to develop vCJD. None of the 66 recipients had themselves donated blood between receiving their transfusion and early 2004 when the UKBS implemented a policy of excluding all donors transfused in the UK since 1 January 1980. It is of note that 41 (62%) recipients were aged over 60 years at the time of transfusion and were not eligible to donate. All living recipients ($n = 26$) have been informed of their risk and advised not to donate blood, tissues or organs. Three instances of probable transfusion transmitted vCJD infection have occurred, including two confirmed clinical cases and one pre- or subclinical infection. Of these, two cases have died, and one is still alive (see succeeding discussion). Figures 1 and 2 show the survival period for dead (transfusion to death) and live recipients (transfusion to 1 March 2006) of vCJD components, respectively, according to the interval between transfusion and onset of clinical symptoms in the donor.

Dead recipients

Forty recipients (61%) are known to be dead, with mean age at death 66 ± 19 years. Table 2 gives the time and cause of death as stated on death certificates for the recipients known to have died. Around half ($n = 21$) of the dead recipients died within a year of receiving their transfusion, with only seven surviving for more than 5 years. Two recipients, who died 4 months and 14 months, respectively, after transfusion had





'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,

Table 2 Cause of death of variant Creutzfeldt–Jakob disease recipients [whose donor has died (*n* = 40)

Interval from transfusion to death	Number of recipients	Cause of death
< 1 month	2	Acute myocardial necrosis Cancer [2] Myocardial infarction Septicaemia [2] Scalds [1 m, 1 adult]
1 – < 6 months	3	Aplastic anaemia Suicide [1 m, 1 adult] Cancer Myocardial infarction [2] Myeloma Renal failure [2] Stroke/cerebrovascular accident/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 Myelofibrosis Disseminated sclerosis Spinal cord glioblastoma
5 – < 10 years	6	Cerebrovascular accident Ischaemic heart disease Acute lymphoblastic leukaemia Carcinoma of pancreatic cancer Peripheral vascular atherosclerosis/COAD[a]
≥ 10 years	2	Bronchopneumonia Ischaemic heart disease

[a]Specified as cause of death on death certificate [2].
[b]Post-mortem biopsy of spleen, tonsil or lymph node + PrP^Sc^ detection [8].

16 months after transfusion and was later diagnosed with neuropathologically confirmed vCJD. Protease-resistant prion protein (PrP^{Sc}) was detected in the spleen and 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 donors

Time elapsed since transfusion[a]	Current age of recipient (years),[a]	Blood component transfused	Interval between donation and onset of clinical symptoms of vCJD in donor[b]
1 – < 2 years	48	Platelets, fresh frozen plasma	−9 m
	49	Red cells, platelets	+9 m
	83	Red cells, fresh frozen plasma, platelets	2 m
2 – < 3 years	38	Red cells, platelets	7 m
3 – < 4 years	58	Red cells, platelets	13 m
	85	Red cells, platelets	22 m
	93	Red cells	+84 m
4 – < 5 years	59	Red cells, platelets	23 m
	67	Red cells, platelets	13 m
	69	Red cells, platelets	23 m
5 – < 6 years	35	Red cells, platelets	14 m
	52	Red cells, platelets	24 m
	64	Red cells, platelets	44 m
	71	Red cells, platelets	13 m
6 – < 7 years	–		–
7 – < 8 years	42	Red cells, platelets	9 m
8 – < 9 years	37	Red cells, platelets	23 m
	74	Red cells, platelets	12 m
	76	Red cells, platelets	16 m
	87	Red cells, platelets	55 m
9 – < 10 years	75	Red cells, platelets	20 m
> 10 years	33	Red cells, platelets, fresh frozen plasma	3 m
	46	Red cells, platelets	14 m
	67	Red cells, platelets	46 m
	70	Red cells, platelets	30 m
	75	Red cells, platelets	24 m
	87	Red cells, platelets	42 m

[a]As at 1 March 2006.
[b]A negative interval denotes that donation was made by individual who [illegible]
[c]Probable variant Creutzfeldt–Jakob disease case [9].

sCJD between 1 and 5 years after donation. Of these, 11 [illegible] received red cell components, eight recipients (46%) [illegible] platelets and one (5%) received fresh frozen plasma.

As of 1 March 2006, 12 recipients are confirmed dead [illegible] a mean age at death of 74 ± 15 years. Of these, five died [illegible] after transfusion (four within a week, and one 2 months) [illegible] and seven survived for between 1 and 5 years after receiving [illegible] their transfusion before dying of a variety of [illegible] related causes (cerebrovascular accident/stroke, n = 3, [illegible] myeloid leukaemia, n = 3, general debility/old age, n = 1). Seven recipients are not known to be dead from [illegible] to date, and are therefore presumed to be alive. The mean age [illegible] of these seven recipients is 56 ± 19 years. The time elapsed [illegible] since their transfusion ranges from 7 to 9 years. The cause of [illegible] a further recipient is unknown. None of the sCJD recipients [illegible] identified as having received blood from donors who went [illegible] on to develop sCJD have appeared on the NCJDSU register [illegible] to date.

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 [illegible] with identifi[illegible]

Case	Transfusion date	Number of donors of labile blood components transfused	Blood components donated to vCJD patient
1	1993	38	Cryoprecipitate [illegible] Fresh frozen plasma [illegible] Platelets [illegible] Red cells [illegible] Whole blood [illegible]
1	1993	65	Cryoprecipitate [illegible] Fresh frozen plasma [illegible] Platelets [illegible] Red cells [illegible]
2	1983	2[b]	Red cells
2	1993	3	Fresh frozen plasma
3	1994	4	Red cells
4[c]	1999	5	Red cells [illegible] [illegible] (3)
5[a]	1996	5[d]	[illegible]
6[a]	1997	1+[e]	[illegible]
7	1982	2[b]	Whole blood

[a]Two of these cases linked to donors already on the National CJD Surveillance Unit [illegible].
[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 [illegible].
[e]One donor already on NCJDSU register as vCJD case. Search for 40+ donors to Case 6 [illegible] 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* [illegible] the individu[illegible] sion transmitted infection was [illegible] indicating that individuals with [illegible] to secondary infection with vCJD [illegible] infected through blood transfus[illegible] of the recipient population are [illegible] ative risk of secondary infection [illegible] genotype is uncertain, a recent [illegible] model suggests that individual[illegible] vCJD, with a frequency of risk fr[illegible] to heterozygote methionine/valine homo[illegible] accelerated incubation backgroun[illegible] that some people will possess [illegible] to infection.

The analysis of vCJD cases w[illegible] further 100 d[illegible] the group who were linked [illegible] transplant [illegible] tested but the donors are the [illegible] lacking DNA and tissue indi[illegible] of this material have been analyz[illegible] donors. To date, none of these [illegible] vCJD, with the exception of the [illegible] clinical cases of vCJD described [illegible]

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4 [illegible]
5 [illegible]

Effectiveness of leucoreduction for removal of [illegible] transmissible spongiform encephalopathi[illegible] from [illegible]

Luisa Gregori, Nancy McCombie, Douglas Palmer, Paul Birch, Samuel O Sowemimo-Coker, An[illegible]

In 1999, the UK implemented universal leucoreduction as a precaution against transmission [illegible] Jakob disease by transfusion of domestic blood or red blood cells. We aimed to assess how [illegible] reduced infectivity of transmissible spongiform encephalopathies (TSEs) in [illegible] 450 mL [illegible] and pooled from scrapie-infected hamsters was leucoreduced with a commercial filter. [illegible] were quantified, and infectivity titres measured. Blood cell recovery and white blood cell [illegible] American Association of Blood Banks standards. Leucofiltration removed [illegible] ID 12) of the [illegible] endogenously infected blood. Leucoreduction is necessary for the removal of [illegible] 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 [illegible] brain homogenate containing [illegible] dose$_{50}$ (ID$_{50}$) of hamster-adapted [illegible] low dose of infectivity was used [illegible] of the inoculum [illegible] blood. The [illegible] approved by the University of [illegible] Animal Care and [illegible] Committee.

We [illegible] one at 106 days and one at 121 [illegible] Under carbon dioxide [illegible] drawn from the [illegible] ventricle [illegible] anticoagulant. [illegible] tissue. Only [illegible] no visible clots were [illegible]

Two [illegible] Corporation, [illegible] Washington, [illegible] for [illegible] [illegible] complete [illegible] of [illegible] complied with [illegible] [AABB] [illegible] and [illegible] for [illegible] The [illegible]

[illegible]

	Volume inoculated (mL)	Total animals inoculated	Total animals infected	Titre in ID/mL (SD)	Fractional distribution of infectivity
Whole blood	5·2	104	50	13·1 (1·6)	1
Leucoreduced blood	5·4	108	34	7·6 (1·2)	0·58

Titre and SD calculated from the Poisson distribution as described in the text.

Table 2: Concentration of TSE infectivity in whole and leucoreduced blood

approached, but did not fully achieve all specifications; furthermore, because more than one filter is involved, more titrations would have been required to evaluate the removal of infectivity.

For the infectivity study, 448·5 mL of CP2D-anticoagulated whole hamster blood was pooled into the whole-blood receiving bag of a Leukotrap WB collection set and processed within the 8-h time limit specified by the AABB. Filtration was done at room temperature under gravity with a 60-inch pressure head on the in-line WBF2 filter, and was completed in 30 min. After removal of a 19 mL sample of the leucoreduced whole blood for subsequent testing, the remainder was centrifuged at 4150 rpm (about 5000 g) for 8 min at room temperature in a Sorvall RC-3C centrifuge. The plasma fraction was expressed into a satellite in-line bag. A preservative and stabiliser, AS3, was added to the red blood cells. Samples of the pre-filtration whole blood, post-filtration whole blood, red blood cells, and plasma were removed for analysis of cell composition and for titration in animals.

Cellular composition of the blood was assessed with a HemaVet five-part differential cell counter calibrated for hamster blood cells (Drew Scientific, Oxford, CT, USA). The residual white blood cell concentrations in the leucoreduced samples were measured by manual count and flow cytometry.

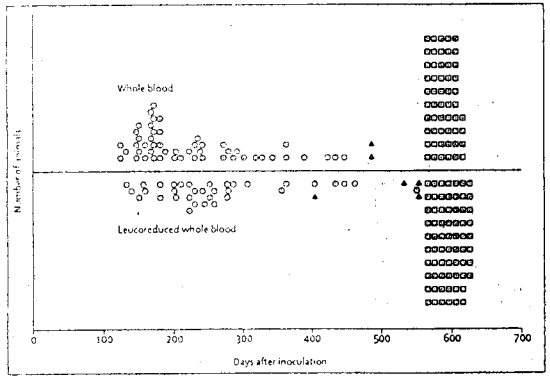

Figure: Incubation times of infections from whole and leucoreduced blood
Results of inoculations of whole blood are represented by data above the horizontal line; those from inoculations of leucoreduced blood are shown below the line. Circles represent infected animals. Squares represent uninfected animals that survived to the end of the experiment. Triangles represent animals that died intercurrently of causes other than the inoculum.

Infectivity of whole and leucoreduced blood was quantified by limiting dilution titration, a method developed in the Rohwer laboratory. The two samples were processed and inoculated separately and sequentially. Each sample of blood was sonicated with a separate sterile probe to lyse cells and disperse infectivity. It was then immediately inoculated intracranially, 50 µl at a time, into about 100 weanling golden Syrian hamsters that were deeply anaesthetised with pentobarbital. Animals were maintained for 566 days; those that contracted scrapie were killed when the clinical diagnosis was conclusive, and animals still alive at the end of the study were killed. All brains were tested for the presence of the proteinase K-resistant form of prion protein by western blot using 3F4 antibody.

The limiting dilution of an endpoint dilution titration is that at which not all of the inoculated animals become infected. At limiting dilution, the distribution of infectivity into individual inoculations is described by the Poisson distribution, where P(0)=probability of no infections at that dilution and inoculation volume, or (1–probability of infection). From the Poisson distribution $P(0)=e^{-titre\times vol}$ and titre=–ln(P[0]) expressed as ID/(inoculation volume). SD of the limiting dilution titre is the square root of the titre in ID/mL divided by the total volume inoculated in mL.

Table 1 shows the distribution of cells in each component of the scrapie-infected blood. Leucofiltration reduced the number of white blood cells by 2·9 log, thereby meeting the AABB standard. White cell contamination of the red blood cell fraction and red blood cell recovery were within AABB specifications of less than 5×10^6 and greater than 85%, respectively. Hamster platelets are not removed by the WBF2 filter, and partition with the red cells during centrifugation.

The incubation times of infections in each measurement are shown in the figure. At limiting dilution, incubation times begin at the end of the predictable dose response seen in endpoint dilution titrations (about 140 days) and rarely extend beyond 500 days. All clinical and western blot results were consistent.

The limiting dilution titre of the whole blood pool (table 2) was close to the values from titrations of similar pools of whole blood by this method (unpublished data). Leucofiltration of whole blood removed only 42% (SD 12) of the initial TSE infectivity (table 2); of the 5900 ID present in the original unit of blood, 3400 ID were recovered in the leucofiltered blood.

Ideally, leucoreduction would be validated by measuring infectivity concentrations before and after leucoreduction of full units of vCJD-infected human blood. However, it is not currently possible to assay either infectivity or the infection-specific form of the prion protein in human blood. By contrast, limiting dilution titration of rodent blood can detect less than 1 ID/mL of TSE infectivity and can readily show a difference of less 20% between samples. With this technique we did a study that: avoided the issue of spikes by using endogenously infected blood; avoided the question of scale by using a human-sized unit of fresh hamster blood obtained within the time limits specified for human blood; minimised the possibility of artefact by using a commercial blood collection set with integral filtration unit and a blood centre centrifuge and expressor; and achieved precision in the infectivity measurements by limiting dilution inoculation of 5 mL of each fraction. We assessed the performance of the filter by measuring the level of white blood cell reduction obtained and the cell recoveries of each component. The leucoreduction met or exceeded AABB specifications for all relevant variables.

Leucoreduction removed only 42% of the initial TSE infectivity from whole blood. This distribution is consistent with that obtained in a centrifugal separation of TSE-infected hamster whole blood, in which the buffy coat contained 70% of the total white cells but only 45% of the total whole blood infectivity (unpublished data). Both methods showed that a substantial proportion of the TSE infectivity was not associated with white cells. We have shown previously[1] that TSE infectivity is not associated with highly purified platelets, and we are currently testing purified red blood cells. We presume that the majority of blood-borne infectivity is plasma-associated.

Although leucoreduction is a necessary step for removing white-cell-associated TSE infectivity from blood, this process is insufficient to remove the risk from an infected transfusion unit. Due to the low concentration of TSE infectivity in blood and the absence of screening or inactivation alternatives, removal is an attractive strategy. However, the feasibility of removal depends upon the actual associations and distributions of TSE infectivity in blood itself, which can only be ascertained by assessment of endogenous blood-borne infectivity.

Contributors
The overall design and execution of [illegible] management of the logistics and all [illegible] by L Gregori and R G Rohwer with the [illegible] of the staff of the Molecular Neurovirology Laboratory. A [illegible] N McCormick, D Palmer, and P Birch supplied expert [illegible] blood collection, component separation [illegible] and quantitation of white blood cells. D [illegible] and P Birch in [illegible] interpreted flow cytometry. S Coker [illegible] experience in the use [illegible] collection set and leucofilter.

Conflict of interest statement
R G Rohwer is a cofounder and part owner [illegible] Diagnostics Technologies, which is developing technologies for the removal of TSE infectivity from blood [illegible] L Gregori receives contract support from Pathogen [illegible] Technologies for studies on TSE removal. Neither [illegible] Pall Corporation, which produces leucofilters and is developing TSE removal strategies for blood. The remaining authors declare that they have no competing financial interests.

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Predicting susceptibility and incubation time of human-to-human transmission of vCJD

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Summary

Background Identification of possible transmission of variant Creutzfeldt-Jakob disease (vCJD) via blood transfusion has caused concern over spread of the disease within the human population. We aimed to model iatrogenic spread to enable a comparison of transmission efficiencies of vCJD and bovine spongiform encephalopathy (BSE) and an assessment of the effect of the codon-129 polymorphism on human susceptibility.

Methods Mice were produced to express human or bovine prion protein (PrP) by direct replacement of the mouse *PrP* gene. Since the human *PrP* gene has variation at codon 129, with MM, VV, and MV genotypes, three inbred lines with an identical genetic background were produced to express human PrP with the codon-129 MM, MV, and VV genotypes. Mice were inoculated with BSE or vCJD and assessed for clinical and pathological signs of disease.

Findings BSE was transmitted to the bovine line but did not transmit to the human lines. By contrast, vCJD was transmitted to all three human lines with different pathological characteristics for each genotype and a gradation of transmission efficiency from MM to MV to VV.

Interpretation Transmission of BSE to human beings is probably restricted by the presence of a significant species barrier. However, there seems to be a substantially reduced barrier for human-to-human transmission of vCJD. Moreover, all individuals, irrespective of codon-129 genotype, could be susceptible to secondary transmission of vCJD through routes such as blood transfusion. A lengthy preclinical disease is predicted by these models, which may represent a risk for further disease transmission and thus a significant public-health issue.

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Introduction

After the identification of variant Creutzfeldt-Jakob disease (vCJD) in 1996,[1] there have been many attempts to estimate the extent of the UK epidemic. Many individuals are likely to have been exposed to bovine spongiform encephalopathy (BSE) material through their diet, however, there have been only 161 cases of the disease in the UK. The predicted total number of future cases has ranged from the low hundreds[2] to hundreds of thousands.[3] However, findings from a retrospective immunocytochemical study that aimed to detect prion protein (PrP) in appendix and tonsil specimens suggested a prevalence of BSE infection of 237 per million people in the UK.[4] DNA sequence analysis of the PrP gene (*PRNP*) in vCJD has shown that 100% of tested cases are homozygous for methionine at the codon-129 polymorphism compared with about 40% of the general white population and about 70% of sporadic CJD cases. The methionine homozygous genotype (MM) has been included as a limiting variable in most mathematical predictions of the size of the epidemic.[5] Identification at autopsy of preclinical vCJD infection in a methionine/valine (MV) heterozygous individual who had received a transfusion of red cells from a donor who later died of vCJD, was the first indication that MM might not be the only susceptible genotype.[6]

Polymorphisms and mutations in *PRNP* in various species can affect disease susceptibility, although the precise mechanisms by which these effects are mediated have not been established.[6,7] Codon 129 of the human *PRNP* gene has been shown to affect the clinicopathological phenotype of disease in CJD and fatal familial insomnia.[8–12] Heterozygosity at *PRNP* codon 129, when compared with homozygous individuals, has been reported to lengthen incubation times in iatrogenic CJD cases associated with growth hormone treatment, and in kuru,[9,14] whereas valine homozygosity (VV) has been proposed to be protective for both BSE and vCJD transmission in studies that used murine models overexpressing human PrP.[15] At a molecular level, the biophysical properties of PrP refolding into the disease associated form (PrP^{Sc}) have been shown to be affected by the codon-129 genotype, with the methionine variant having an increased propensity to form PrP^{Sc}-like structures.[16]

We sought to analyse the transmission characteristics of BSE and vCJD to four inbred lines of transgenic mice after intracerebral inoculation with brain homogenate from cases of vCJD and BSE. We then aimed to use these models to address the apparent low level of vCJD in the human population resulting from exposure to BSE and to predict the potential for human-to-human spread of vCJD and the susceptibility of different genotypes in the human population.

Methods

Transgenic mice

Details of how the gene-targeted transgenic lines were created are supplied as supplementary information

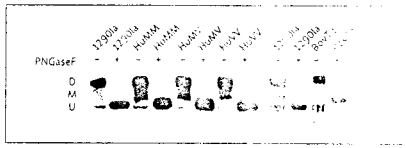

Figure 1: Western blot of brain extract from uninoculated mice showing that PrP^{C} is detected with equivalent electrophoretic mobility and glycosylation ratio in all three human transgenic lines
D=diglycosylated PrP^{C} band; M=monoglycosylated PrP^{C} band; U=unglycosylated PrP^{C} band. In the BovTg line, a deglycosylated band is detected of increased molecular weight due to the additional N-terminal octapeptide repeat. Protein levels are similar to the wild-type line used in generating the transgenic (129Ola). Glycosylation is confirmed by the reduction to a single band after deglycosylation with the enzyme PNGaseF. The anti-PrP antibody 7A12 was used for the HumTg blot as it will react with both murine and human PrP, and was used for the BovTg blot.

See Online for webappendix

(webappendix). Transgenic mice were anaesthetised with halothane and then injected with 0·02 mL of 10% homogenate into the right cerebral hemisphere. The vCJD tissue homogenate (at 10^{-2} dilution) was supplied by the UK National Institute for Biological Standards and Control (Code NIHBY0/0003). BSE-infected cattle brain (Veterinary Laboratories Agency, reference 12/92) was prepared by maceration of the tissue in phosphate saline to a dilution of 10^{-1}. From 100 days they were scored each week for signs of disease.[17] Mice were killed by cervical dislocation whether they had clinical signs

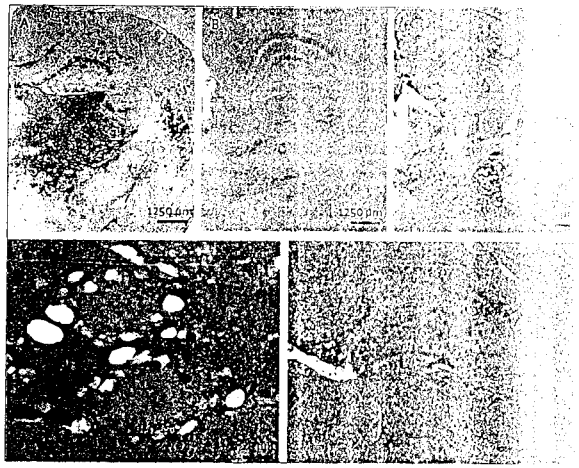

Figure 2: Immunocytochemistry of histological sections with anti-PrP antibody 6H4 showing the cortical, hippocampal, and thalamic regions of the mouse brain with PrP detection (brown)
A–D: Human transgenic mice with vCJD inoculum. A: HuMM mouse 693 days post inoculation. B: HuMM mouse 707 days post inoculation. C: HuVV mouse 693 days post inoculation. D: Florid plaques found in the hippocampus of the HuMM mouse in panel A. Each plaque has an eosinophilic core with a paler halo and is surrounded by a zone of vacuolation (haematoxylin and eosin stain). E: Hippocampal region of a BovTg mouse inoculated with BSE, PrP is deposited in a more diffuse/granular form with occasional plaques.

0·5% w/v sodium deoxycholate, 0·9% w/v sodium chloride, 50mM Tris-HCl pH 7·5) to give a 10% suspension. This material was cleared by centrifugation and the supernatant digested with PNGaseF. The products were denatured then loaded onto a 12% Novex Tris/Glycine gel (Invitrogen, UK). After electrophoresis the gel was blotted onto PVDF membrane. PrP was identified with the SuperSignal West Dura chemiluminescence detection kit (Pierce, UK) with primary antibody 8H4[24] at 1:20000 and an anti-mouse IgG peroxidase-linked secondary (Jackson Immuno Research Laboratories, UK) at 1:10000. Images were captured on radiographic film and with a Kodak 440CF digital imager (figure 1).

Role of the funding source

The sponsors of this study had no role in study design, data collection, data analysis, data interpretation, or writing of the report. The corresponding author had full access to all the data in the study and had final responsibility for the decision to submit for publication.

Results

We first investigated the potential effects of the species barrier between BSE and human beings and any alteration in that barrier once BSE had passed through people in the form of vCJD. We then investigated the effect of the codon-129 polymorphism on human-to-human transmission of vCJD using gene-targeted inbred mice developed by direct replacement of the murine *PrP* gene for the human gene. These mice produce PrP under the control of the normal regulatory elements for PrP and thus express physiological concentrations of PrP with the correct tissue distribution (figure 1). Three inbred lines with an identical genetic background were produced to express human PrP with the codon-129 MM, MV, and VV genotypes (designated HuMM, HuMV, and HuVV, respectively). Each line differs by only a single codon in *PRNP* and in all other respects the mice were genetically identical. Additionally, in an identical manner, we produced mice that express bovine PrP to enable direct comparisons to be made not only between transgenic and wild-type mice, but also between each of the transgenic lines.

Typical clinical signs of TSE disease were seen in more than half (15/22) the BovTg mice inoculated with BSE material with a mean incubation period of 551 days (SD 47). These clinical cases were confirmed by a positive test for the presence of TSE vacuolation or PrP^{Sc} deposition by immunocytochemistry. The lesion profiles generated for targeting and degree of vacuolation showed similar patterns for all positive mice. Immunocytochemical data showed PrP^{Sc} deposition mainly in a diffuse and synaptic form, and also as plaque-like structures, frequently associated with areas of spongiform change (figure 2). Deposition was most abundant in the thalamus and hippocampus, but was recorded throughout other regions of the brain. The cerebral cortex showed only occasional plaque-like structures and the cerebellum had only a few areas of PrP^{Sc} deposition limited to the granule cell layer. Further pathological analysis was undertaken on mice that were culled for reasons other than clinical TSE (intercurrent deaths). This analysis showed that all the brains had pathological signs of TSE disease in terms of vacuolation or PrP deposition. Thus, all the bovine transgenic mice (22/22) seemed to be susceptible to BSE infection, although not all developed clinical signs of infection (tables 1 and 2).

HuMM, HuMV, and HuVV mice were inoculated with BSE material and after extensive pathological analysis all were confirmed as negative for TSE transmission (table 1). Mice of each genotype line were inoculated with vCJD material. Two pathologically confirmed clinically positive mice were seen in the HuMM line (at 497 and 630 days post inoculation), one in the HuMV line (at 665 days post inoculation), and none in the HuVV line (table 3). HuMM mice were more likely to show disease-associated vacuolation, beginning at around 560 days post inoculation. Six were scored positive and showed similar distribution of vacuolation in the brain, with the highest levels found in the dorsal medulla, thalamus, and cerebellar white matter. By contrast, only a single mouse in each of the HuMV and HuVV groups scored positive for vacuolation at approximately 700 days post inoculation.

Most of the HuMM mice (11/15) showed PrP^{Sc} deposition in most areas of the brain at a relatively early stage (from around 370 days post inoculation), before the vacuolar pathology became evident. From 500 days post inoculation the appearance of vacuolation was accompanied by a significant increase in PrP^{Sc} deposition. By contrast, although PrP^{Sc} deposition was identified in many HuMV mice (11/13), they had little deposition, restricted to only a few areas (including the ventrolateral and ventromedial thalamic nuclei and the red nucleus of the mid-brain), even after 700 days post inoculation

	Clinically positive	Vacuolation positive	PrP positive	Negative*
HuMM (n=17)				
0–400	0	0	2	2
401–500	1	1	1	2
501–600	0	1	3	2
>600	1	4	5	0
HuMV (n=16)				
0–400	0	0	0	0
401–500	0	0	0	0
501–600	0	0	4	3
>600	1	1	7	2
HuVV (n=16)				
0–400	0	0	0	0
401–500	0	0	0	1
501–600	0	0	0	5
>600	0	1	1	9

*Negative by clinical or pathological analysis, or positive by clinical scoring but not confirmed by pathology.

Table 3: **Clinical and pathological scoring of human transgenic mice, by number of days after vCJD inoculation**

	HuMM	HuMV	HuVV
Vacuolation*	Thalamus (severe), cerebral cortex and hippocampus (mild), cerebellar cortex (minimal)	Thalamus, cerebral cortex and cerebellar	
Plaque formation*	Fibrillary amyloid plaques, florid and non-florid plaques in cerebral cortex and hippocampus; no evidence of plaques in cerebellum	No evidence of	
PrP deposition†	Intense staining of plaques in hippocampus and cerebral cortex; plaque-like, pericellular, and amorphous deposits in the hippocampus; synaptic, peri-neuronal, and diffuse perivascular deposits in the thalamus	Occasional	

*Analysed with haematoxylin and eosin staining. †Analysed with immunocytochemical techniques.

Table 4: **Comparison of TSE-associated neuropathology in human transgenic mice**

Discussion

Although the cattle BSE epidemic in the UK has amounted to more than 180 000 cases since the 1980s, the extent of the human vCJD epidemic has so far remained limited with the total number of cases worldwide currently at 190. One explanation for this apparent discrepancy is that there exists a significant species barrier between cattle and human beings, which limits the susceptibility of the human population to BSE. The data shown here suggest that this could indeed be the case since BSE was readily transmissible to the bovine transgenic mice but not to the human transgenic mice. However, once BSE has passed through human beings in the form of vCJD, the transmissibility of this TSE strain is altered for the human population.

All the human transgenic lines inoculated with BSE were negative for TSE transmission which suggests that either the human transgenic lines are relatively resistant to transmission of BSE or the incubation time is longer than the lifespan of the experimental (approximately 700 days). BSE transmission previously observed by others, in human transgenic lines overexpressing the human prion protein, could be due to overexpression of the *Prnp* gene and may not therefore give a true reflection of the species barrier between BSE and human beings.[16,17] This apparent resistance of human transgenic mice to BSE could be explained by a large species barrier and this in turn could explain the low number of vCJD cases in the human population.

vCJD was transmitted to all three human lines with different pathological characteristics for each genotype, and a gradation of transmission efficiency from MM to MV to VV. The greater transmission efficiency in HuMM mice suggests that homozygosity for methionine at codon 129 leads to earlier onset of TSE-related pathological features and clinical disease than for the other two genotypes. The differences in PrP^{Sc} deposition in the HuMM and HuMV lines suggest that the codon-129 polymorphism in human beings is likely to affect the distribution of PrP^{Sc} deposition in the brain. Moreover, the similar numbers that scored positive for PrP^{Sc} deposition in each of the MM and MV groups (11/15 and 11/13 respectively) suggest that the two genotypes might be equally susceptible to vCJD, but with different incubation periods. Titration experiments are needed to fully compare the susceptibility of each line. The single HuVV mouse positive for PrP^{Sc} shows that VV individuals may be susceptible to vCJD with very long incubation times, including a lengthy subclinical phase. Transmission studies from all three genotype mice are now underway to examine the infectious nature of the disease and determine any alterations in the strain characteristics on passage through human transgenic mice. By contrast with published data suggesting that VV individuals cannot propagate the vCJD biochemical phenotype,[4] the data presented here suggest that the PrP^{Sc} type will remain a useful diagnostic feature of secondary vCJD infection irrespective of codon-129 genotype, as has been observed for the two extant cases of transfusion-associated vCJD infection.[5,27]

Transmission of vCJD to the three lines of human transgenic mice indicates that the human population could be at significantly heightened risk of developing disease after iatrogenic exposure to vCJD. Secondary transmission of vCJD has partly removed the cattle-to-human species barrier and has resulted in an agent that can be transmitted from human to human with relative efficiency. Transmission studies in cynomolgus macaques provide further evidence for this agent adaptation as they show reduction in incubation times after serial passage of BSE.[28] Our BSE inoculation at 10^{-1} dilution was compared with vCJD inoculation at 10^{-2} because the latter inoculum was found to be toxic to the mice at 10^{-1}. Use of a higher dose of vCJD inoculum would have maintained or increased the transmission efficiency of vCJD and enhanced the current findings.

Our findings raise concerns relevant to the possibility of secondary transmission of vCJD through blood transfusion, fractionated blood products, or contaminated surgical instruments. For this study mice were injected intracerebrally, whereas the probable human exposure to these agents is by peripheral routes (eg, oral or intravenous), and thus human-to-human exposures might be significantly less efficient. However, it is difficult to know for sure what the practical implications might be in human beings. Peripheral route challenge is in progress; however, BSE transmission studies in primates have shown the intravenous route to be as efficient as the intracerebral route, with an extension of the incubation time.[28]

Although all cases of vCJD up to now have been observed in the MM genotype, this model of human-to-human vCJD transmission suggests that other genotypes are also susceptible. In our experimental setting, all *PRNP* codon-129 genotypes are susceptible to vCJD infection; however, progressive development of pathological TSE features (vacuolation and PrP deposition) is more rapid in the MM-genotype mice. An explanation for this finding might be provided by in-vitro conversion of recombinant human PrP by BSE and vCJD agents, which has shown that PrP with methionine at position 129 is more efficiently converted than PrP with valine, and that conversion by vCJD is significantly more efficient than by BSE.[29] Long incubation periods during which PrP^{Sc} is deposited predicts that, in human beings, infection could be present in all genotypes for a significant period before clinical onset. Incubation periods of more than 30 years have been reported in the human TSE disease kuru.[30]

The possibility that an MV or VV genotype could result in a phenotype distinct from that recognised in vCJD draws attention to the importance of systematic assessment of the clinical, genetic, pathological, and biochemical features of all human prion diseases. [illegible] findings indicate that for human-to-human [illegible] infection it should be assumed that all [illegible] genotype individuals (not just MM) can be infected, [illegible] long incubation times can occur, and that a significant level of subclinical disease might be present in the population.

Contributors

MTB, PH, and CP did immunocytochemical and western blot analyses. JCM, NT, HNB, and LA produced the transgenic mouse lines. JWI supplied vCJD case material and reviewed the neuropathology. NT did the mouse inoculations; and MTB, PH, MWH, RGW, JWI, and JMS prepared the manuscript.

Conflicts of interest

We have no conflicts of interest.

Acknowledgments

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Prion diseases are efficiently transmitted by blood transfusion in sheep

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The emergence of variant Creutzfeld-Jakob disease, following on from the bovine spongiform encephalopathy (BSE) epidemic, led to concerns about the potential risk of iatrogenic transmission of disease by blood transfusion and the introduction of costly control measures to protect blood supplies. We previously reported preliminary data demonstrating the transmission of BSE and natural scrapie by blood transfusion in sheep. The final results of this experiment, reported here, give unexpectedly high transmission rates by transfusion of 36% for BSE and 43% for scrapie. A proportion of BSE-infected tranfusion recipients (3 of 8) survived for up to 7 years without showing clinical signs of disease. The majority of transmissions resulted from blood collected from donors at more than 50% of the estimated incubation period. The high transmission rates and relatively short and consistent incubation periods in clinically positive recipients suggest that infectivity titers in blood were substantial and/or that blood transfusion is an efficient method of transmission. This experiment has established the value of using sheep as a model for studying transmission of variant Creutzfeld-Jakob disease by blood products in humans. (Blood. 2008;112: 4739-4745)

Introduction

Transmissible spongiform encephalopathies (TSEs) are neurodegenerative diseases, which include Creutzfeld-Jakob disease (CJD) in man, scrapie in sheep and bovine spongiform encephalopathy (BSE) in cattle. A new variant of CJD (termed vCJD) was recognized in the United Kingdom in the mid-1990s, apparently as a result of transmission of BSE to humans.[1] To date, there have been 165 cases of vCJD recorded in the United Kingdom, as well as several cases in other countries. Human TSEs are characterized by long asymptomatic incubation periods (usually several years), and there is no reliable test for detecting infection before the onset of clinical disease. It is not known how many people in the United Kingdom harbor vCJD, although estimates based on screening of tonsil and appendix samples suggest there could be up to 4000.[2] These infected persons pose a risk of human-to-human transmission via blood transfusion or contaminated surgical instruments.

In patients with vCJD, there is widespread replication of the infectious agent and deposition of PrP^{Sc} (disease-associated form of prion protein) in lymphoreticular tissues, such as the tonsil, spleen, and lymph nodes, in contrast to sCJD, where lymphoreticular involvement is minimal.[3] The fact that lymphocytes continuously recirculate between blood and lymphoreticular tissues strongly suggests that the blood of vCJD patients is probably infectious. Data from rodent TSE models had shown that the highest levels of infectivity in blood were associated with leukocytes and, to a lesser extent, plasma.[4] As a result, costly control measures such as leukodepletion (filtration of blood and blood products to remove leukocytes) and importation of plasma were introduced to protect United Kingdom blood supplies, despite the limited data that were then available to judge the size of the risk and the efficacy of the control measures.

The potential for using sheep as a model for studying the risks of vCJD transmission by blood transfusion was highlighted by the similarity between the distribution of infectivity and PrP^{Sc} in sheep infected with TSEs and humans infected with vCJD.[5-7] One factor limiting the successful transmission of TSEs by blood in rodent models was the small volumes of blood that could be injected. In contrast, the relative similarity in size of sheep and humans means that volumes of blood comparable with those used in human transfusion practice can be collected from and transfused into sheep. Using this model, we previously reported preliminary results showing that both BSE and natural scrapie could be transmitted between sheep by blood transfusion.[8,9] Although scrapie is not thought to be transmissible to humans, it was included as a representative of infection acquired under field conditions, which may give different results to those obtained from experimentally infected animals. Our blood transfusion experiment in sheep is complete after 9 years, and this paper presents the full data from the study. The overall transmission rates for both scrapie and BSE are surprisingly high when factors such as the stage of infection and genetic background are taken into account, suggesting that blood transfusion represents an efficient route of transmission.

Methods

Donor and recipient sheep

The animal work was reviewed and approved by internal ethical review procedures at the Institute for Animal Health, United Kingdom, and carried out under the authority of Home Office Project Licences.

PrP genotypes of all sheep were confirmed by sequencing the coding region of the *PrP* gene[10] and are represented by single letter amino acid code for codons 136, 154, and 171, which have been linked to scrapie susceptibility (eg, ARQ represents alanine, arginine, and glutamine, respectively, at codons 136, 154, and 171).

All donor sheep were from the Edinburgh NPU Cheviot flock, which has endemic natural scrapie. The recipient sheep (including scrapie negative control donors) were Cheviots derived from the DEFRA scrapie-free (DEFRA/SF) flock of New Zealand origin. Transfusion recipients and positive and negative controls were housed in a purpose-built isolation unit on a different site to the donors, with strict procedures in place to minimize the risk of cross-contamination between groups, as described.[9] The sheep were scored at weekly intervals for clinical signs of TSEs and killed when they reached humane endpoints agreed with the Home Office. For experimentally inoculated animals (BSE donors, positive controls, and transfusion recipients), the incubation period (IP) in clinically positive sheep was defined as the period between the date of inoculation and the date of death. For scrapie-exposed donors, the IP in clinically positive sheep was defined as the age at death (ie, they were assumed to have become infected immediately after birth).

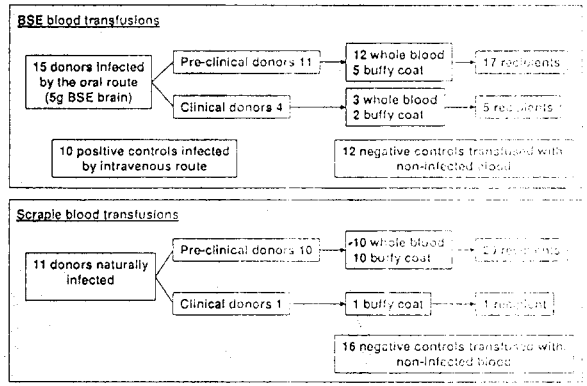
Blood collection and transfusion

Procedures for blood collection/transfusion were as previously described.[9] Briefly, venous blood (450-500 mL = 1 unit) was collected into sterile collection bags (NBPI-Fresenius, Emmer-Compascuum, The Netherlands) containing citrate phosphate dextrose adenine solution as anticoagulant. From donors that were about to be killed, 2 units was collected just before postmortem, whereas from donors that were to be left alive, separate collections of 1 unit were made at least 28 days apart. However, for practical reasons, it was not always possible to collect 2 units of blood from every donor sheep. In most cases where 2 units of blood was obtained, one was transfused as whole blood (without leukodepletion) and the other was used to prepare a buffy coat fraction.

BSE blood transfusions

Fifteen sheep experimentally inoculated either orally (n = 14) or intracerebrally (n = 1) with 5 g or 0.05 g, respectively, of BSE-infected cattle brain homogenate were used as blood donors. The donor *PrP* genotypes were ARQ/ARQ (n = 3), ARQ/AHQ (n = 5), or AHQ/AHQ (n = 7), which are resistant to natural scrapie in the NPU flock but produce the shortest IPs after inoculation with BSE. Two sheep previously reported as donors[9] were excluded from the study (along with their recipients) when regenotyping showed them to be ARQ/ARR and VRQ/AHQ, respectively, genotypes that result in relative resistance to oral infection with BSE.

Eleven donor sheep provided blood for transfusion at the preclinical stage of infection. Eight of these were culled at the time of donation as part of a separate [illegible] 3 preclinical donors went on to [illegible] respective IPs of 625, 761, and 2131 [illegible] used as blood donors to be they [illegible] 561 to 671 days after infection. PrP[illegible] tissues were confirmed in all clinicals [illegible] try (IHC). In 2 donors culled at the [illegible] found in only one tissue in each sheep [illegible] ganglion (at 8 × 49). However, a negative [illegible] tissues were immunostained in another [illegible] recipients of whole blood and 7 [illegible] BSE-infected donors. Figure 1 gives [illegible] whereas details of the donor and recipient [illegible]



Scrapie blood transfusions

The donors for this experiment were [illegible] Cheviot sheep from the Edinburgh NPU [illegible] genotypes of low incidence in the [illegible] pathogenesis studies and [illegible] Blood collections were made [illegible] (2%-25% of [illegible] preclinical [illegible] were culled after the clinical [illegible] 185, or 196 days [illegible] were [illegible] IHC. Two donors were [illegible] 1356 days of age, [illegible] IHC. Two donors [illegible] negative, [illegible] of [illegible] tissues were [illegible] There were 21 scrapie-naive (all VRQ/VRQ) [illegible] scrapie-exposed donors, 11 were [illegible] whole blood. See Figure 1 for a summary [illegible] Table 2 for details of donor and recipient [illegible]

Positive and negative controls

Seven ARQ/AHQ and 2 ARQ/ARQ sheep were [illegible] 0.2 g of the same BSE-infected cattle brain [illegible] the blood donors and served as positive controls [illegible] used in the scrapie transfusion experiment. In the [illegible] transfusion experiment, 12 ARQ/ARQ recipients [illegible] whole blood (n = 6) + buffy coat (n = 6) [illegible] (6 ARQ/AHQ, 1 ARQ/ARR). Two recipients [illegible] after transfusion, respectively, and the remaining [illegible] between 2442 and 2550 days after transfusion. [illegible] scrapie experiment, 10 VRQ/VRQ sheep were [illegible]

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Table 1. Outcome of transfusions from BSE-exposed donor sheep

Donor sheep details								Recipient sheep details				
Donor sheep ID	Donor genotype	Clinical status at donation	Percentage of actual or average incubation period at donation*	Clinical outcome	IHC result	Incubation period, d	Component transfused	Recipient sheep ID	Recipient *PrP* 168 codon genotype	Clinical outcome	IHC result	Incubation period, d
58x51	ARQ/AHQ	Preclinical	12	+	+	2131	WB	D529	PP	+†	–	–
60x42	ARQ/ARQ	Preclinical	22	–	+/–	–	WB	D433	PL	–	–	–
			44		(DRG)‡		WB	F14	PL	–	–	–
J2747	ARQ/AHQ	Preclinical	42	–	–	–	BC	F182	PP	–	–	–
			44				WB	F181	PP	–	–	–
61x24	ARQ/AHQ	Preclinical	42	–	–	–	BC	F238	PP	+†	–	–
			43				WB	F234	PP	–	–	–
J2746	AHQ/AHQ	Preclinical	45	–	–	–	WB	F19	PP	+	+	536
J2353	AHQ/AHQ	Preclinical	51	+	+	629	WB	D505	PP	+	+	610
58x81	ARQ/AHQ	Preclinical	61	–	+/– (IPP)‡	–	BC	D358	PP	–	–	–
58x26	ARQ/AHQ	Preclinical	61	–	–	–	WB	D421	PP	–	–	–
			61				BC	D384	PP	–	–	–
58x27	AHQ/AHQ	Preclinical	61	–	–	–	WB	D452	PP	–	+§	–
			61				BC	D318	PP	–	–	–
58x33	ARQ/AHQ	Preclinical	62	–	–	–	WB	D337	PP	–	+‖	–
			62				WB	D386	PP	–	–	–
J2403	AHQ/AHQ	Preclinical	86	+	+	761	WB	D341	PP	–	–	–
J2721	AHQ/AHQ	Clinical	100	+	+	561	BC	G61	PL	–	+	–
J2727	AHQ/AHQ	Clinical	100	+	+	589	WB	G74	PP	+	+	594
60x69	AHQ/AHQ	Clinical	100	+	+	660	WB	G78	PP	+	+	556
							BC	G49	PP	+	+	531
D389	ARQ/ARQ	Clinical	100	+	+	671	WB	G92	PL	–	+	–

WB indicates whole blood; BC, buffy coat; DRG, dorsal root ganglion; IPP, Ileal Peyer patch; +, positive; and –, negative.

*Calculated from the days after infection at the time of donation, as a percentage either of the final incubation period (in sheep kept alive until the development of clinical signs) or of the average incubation period in orally infected donors (640 days), excluding the outlying incubation period of 2131 days (58x51).

†No evidence of infection was found on postmortem examination of tissues from these clinical suspects; therefore, it is most likely they were clinically misdiagnosed.

‡These tissues were initially scored weakly positive by IHC, but the results were not reproducible in two laboratories and can therefore be considered as inconclusive.

§This sheep died of unrelated causes (ie, without showing clinical signs of BSE) at 1139 days after transfusion but was positive by IHC.

‖This apparently healthy sheep was culled 3018 days after transfusion and found to be positive by IHC; however, further analysis suggested this was a case of "atypical" scrapie and therefore unlikely to be transfusion related.

buffy coat (n = 8) collected from 8 uninfected VRQ/VRQ donors. There were 2 intercurrent deaths at 297 days and 464 days after transfusion, and the other 14 animals were culled between 2052 and 2409 days after transfusion. None of the negative controls for the BSE or scrapie experiments showed clinical signs of TSEs, and all were IHC negative for PrP^{Sc}.

PrP^{Sc} detection by immunohistochemistry

Tissue samples from the brain, spleen, mesenteric lymph node, and palatine tonsil of the sheep under study were fixed in formaldehyde and processed according to standard procedures. Sections were immunolabeled for PrP^{Sc} detection by IHC with primary antibody R145, which recognizes the 222-126 amino acid sequence of ovine PrP,[11] as described previously.[12,13]

Results

BSE transfusion experiment

A total of 5 transfusion recipients showed clinical signs of TSEs and were confirmed positive by IHC and/or Western blot (Table 1; Figure 2). These included 2 (F19 and D505) of 12 sheep transfused with whole blood from donors in the preclinical phase of infection (at ~5% and 59% of estimated IP, respectively), as reported previously.[5,9] Two of 3 recipients of whole blood and one of 2 recipients of buffy coat from donors clinically affected by BSE developed clinical BSE. The IPs in the 5 clinically positive recipient sheep ranged from 531 to 610 days after transfusion (mean ± standard deviation [SD] = 565 ± 35 days), and there was no obvious difference in the IPs of those that received blood from preclinical or clinical donors.

One recipient (D452) of whole blood from a preclinical donor died of unrelated causes at 1139 days after transfusion but had PrP^{Sc}-positive IHC labeling in brain and other tissues. One of 3 recipients of whole blood (G92) and one of 2 recipients of buffy coat (G61) from clinical donors showed weak PrP^{Sc} deposition in the brain and lymphoid tissues after being culled at 2003 and 2497 days after transfusion, respectively, in the absence of clinical signs. Full sequencing of the *PrP* gene of these sheep revealed that they carried an additional proline (P) to leucine (L) substitution at codon 168,[14,15] which appears to be associated with the prolonged survival of these infected sheep. The polymorphism was also identified in 2 recipients of blood from a preclinical BSE-challenged donor, neither of which showed evidence of infection.

Taking the results for all 22 recipients of blood from BSE-exposed donors, 5 clinical cases and 3 sheep showing evidence of infection in the absence of clinical signs were identified, giving an overall transmission rate of 36%.

One recipient was culled for health reasons at 1444 days after transfusion, 2 were culled with suspected TSE clinical signs at 2480 and 2160 days after transfusion, respectively, and the remaining clinically negative sheep were culled between 2239 and 3068 days after transfusion. With one exception, examination of the tissues by IHC did not find evidence of infection. The exception (D337) was culled at 3018 days after transfusion and showed

Table 2. Outcome of transfusions from scrapie-exposed donor sheep

Donor sheep details								Recipient sheep details				
Donor sheep ID	Donor genotype	Clinical status at donation	Percentage of actual or average incubation period at donation*	Clinical outcome	IHC result	Incubation period, d	Component transfused	Recipient sheep ID	Recipient *PrP* 168 codon genotype	Clinical outcome	IHC result	Incubation period, d
67x42	VRQ/VRQ	Preclinical	17	–	–	[illegible]	WB	[illegible]	[illegible]	–	–	[illegible]
			19									
66x45	VRQ/VRQ	Preclinical	17	–	–		WB			–	–	
			19									
67x23	VRQ/VRQ	Preclinical	18	–	–	[illegible]	WB	[illegible]	[illegible]	–	–	[illegible]
			20									
65x13	VRQ/VRQ	Preclinical	28	–	–	[illegible]	WB	[illegible]	[illegible]	–	–	[illegible]
			30									
65x02	VRQ/VRQ	Preclinical	34	–	–		WB			–	–	
			37									
65x03	VRQ/VRQ	Preclinical	34	–	–		WB			–	–	
			37									
61x75	VRQ/ARQ	Preclinical	53	–	–	[illegible]	WB	[illegible]	[illegible]	–	–	[illegible]
			57									
61x68	VRQ/VRQ	Preclinical	64	–	–	[illegible]	WB	[illegible]	[illegible]	–	–	[illegible]
			69									
61x66	VRQ/VRQ	Preclinical	92	–	NC		WB			–	–	
			94									
59x27	VRQ/VRQ	Preclinical	79	–	–	[illegible]	WB	[illegible]	[illegible]	–	–	[illegible]
			82									
59x28	VRQ/VRQ	Clinical	100	–	–	[illegible]	WB	[illegible]	[illegible]	–	–	[illegible]

+ indicates positive; and –, negative.

*Calculated from the age at the time of donation, as a percentage either of the final incubation period (in sheep kept alive until the development of clinical signs) or of the average incubation period (1296 days) for sheep that died or were culled before developing clinical signs.

†No evidence of infection was found on postmortem examination of tissues from these clinical suspects; therefore, it is most likely they were clinically misdiagnosed.

positive PrP^{Sc} labeling in the brain, but with a pattern distinct from that observed in other BSE-infected sheep. The brain PrP^{Sc} distribution involving major white matter tracts and sparing the dorsal motor nucleus of the vagus was similar to that of No. 8 (or "atypical" sheep scrapie) and therefore doubtful to be transfusion-related. No other sheep in the present study showed evidence of being infected with atypical scrapie.

Of the 10 sheep that were infected intravenously with BSE as positive controls, 8 developed clinical signs confirmed by IHC, with an average IP of 702 days (± 61 days, SD). The remaining 2 animals were culled at 2591 days after infection and, although not demonstrably clinically affected, IHC showed PrP^{Sc} deposition in the brains and lymphoid tissues of both animals. These 2 sheep were heterozygous (PL_{168}) for the *PrP* polymorphism P168L, whereas the other 8 were homozygous (PP_{168}).

The PrP^{Sc} profile obtained by IHC from BSE-positive recipients was the same as that found in the orally inoculated donors and in the positive controls.[16] In addition, characteristic BSE glycoform patterns were obtained by Western blot analysis of PrP^{Sc}-positive donor and recipient sheep (data not shown),[9] and inoculation of brain homogenates from infected donors and recipients into a panel of inbred mouse strains produced IPs and lesion profiles characteristic of BSE (data not shown). Taken together, these results confirm that the strain characteristics were not altered after transmission via blood.

Scrapie transfusion experiment

Four of 10 recipients of whole blood and 4 of 10 recipients of buffy coat from donors in the preclinical phase of scrapie infection developed clinical signs of scrapie, which were confirmed by positive IHC results. One sheep transfused with buffy coat from the single clinical donor was also clinically affected and IHC positive

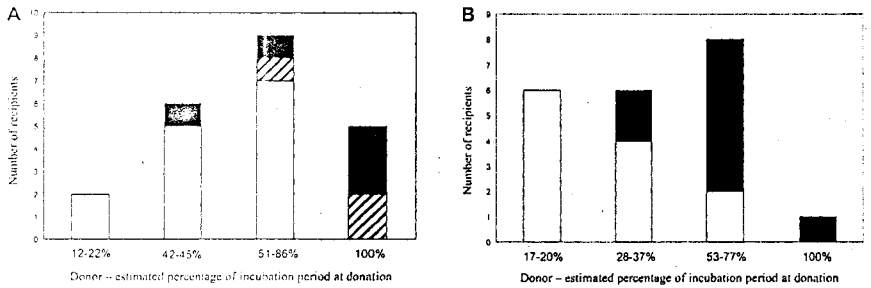

Figure 2. Outcome of transfusions as a function of the stage of disease incubation in the donor. (A) BSE-infected donors. (B) Scrapie-infected donors. For each stage of infection in the donor sheep, the number of uninfected (□), clinically positive/ IHC-positive (■), and clinically negative/IHC-positive (▩) recipients are shown.

transmitted between sheep by blood transfusion, using volumes similar to those used in human transfusions. The overall transmission rates (percentage of all recipients that became infected) were 36% for BSE and 43% for scrapie. For BSE, the figure was much higher than anticipated because 3 of the 8 BSE-infected recipients survived for long periods without showing clinical signs, whereas all the scrapie-infected recipients identified by IHC were also clinically positive. The greater probability of subclinical infection in recipients of blood from BSE-exposed donors is largely the result of variability in the genetic susceptibility to infection among sheep used in the BSE experiment, which will be discussed in "Effect of genetic variation in susceptibility." The results are consistent with the known facts about transmission of vCJD by blood transfusion in humans.[17] Sixty-six patients known to have received labile blood products from 18 donors who subsequently developed vCJD were followed up in an ongoing study. Three of these recipients have been confirmed clinically and pathologically as vCJD cases, with intervals between transfusion and the development of clinical signs ranging from approximately 6.5 years to 8.5 years.[18-20] Another patient, who died of unrelated causes 5 years after transfusion, showed PrP^{Sc} deposits in lymphoid tissues but not brain postmortem, and is thought to represent preclinical or subclinical infection.[21] These 4 patients represent 6% of the total recipients, or 12.5% of recipients surviving longer than 5 years.

Various factors influence the transmission rate by transfusion in both sheep and humans, including: (1) the interval between blood donation and the onset of clinical signs in the donors, (2) genetic variation in susceptibility of donors and recipients, and (3) the blood component transfused.

Stage of incubation period of the donors at the time of blood donation

The effect of the stage of incubation can best be deduced from the results of the scrapie transfusion experiment because the *PrP* genotype of the sheep used (VRQ/VRQ) renders them almost 100% susceptible to natural and experimental infection.[22] The stage of incubation of the donor has a strong influence on the probability of transmission to the recipient (Figure 2). When donations were made at less than or equal to 20% of the estimated IP, there was no disease transmission, whereas donations made at more than 50% of the estimated IP produced an 80% transmission rate, with a mean IP of 729 days (± 99, SD) in the recipients. Blood collected at 28% to 37% of the estimated IP transmitted infection at a lower rate of approximately 33%, and with longer IPs in the recipients of more than 1000 days. The data are consistent with a gradual increase in infectivity in the blood, from approximately 30% to 50% of IP until the clinical phase.

In the BSE transfusion experiment, the correlation between stage of infection and transmission is not clear-cut but shows the same general trend of increasing probability of transmission to recipients as infection progresses in the donors (Figure 2). Possible explanations for the lower transmission rates from preclinical BSE-infected blood donors compared with preclinical scrapie-infected donors include the following:

(a) Variation in susceptibility to infection of both donor and recipient sheep.

(b) Differences in the pathogenesis of natural scrapie and experimental BSE. VRQ/VRQ sheep naturally infected with scrapie have detectable PrP^{Sc} deposits in lymphoid tissues early after infection (ie, < 50% estimated IP).[23,24] Time course studies of ARQ/ARQ sheep orally infected with BSE showed that PrP^{Sc} was not consistently detected in lymphoid tissues before at least 65% of the average IP.[7] If infectivity in blood correlates with its presence in lymphoid tissues, this could explain the differences observed in the 2 transfusion experiments.

The probability of transmission from preclinical donors is of greatest relevance to the human situation. In the case of the 4 transfusion-related transmissions of vCJD, the donors developed clinical signs between 17 and 42 months after donation. The mean IP for vCJD has been estimated to be 16.7 years, with a lower 95% confidence interval of approximately 12.4 years.[25] Therefore, it is probable that the transfusion-related vCJD cases resulted from donations made at least halfway through the IP, which is in agreement with the data from the sheep experiments. In vCJD cases, the timing of detectable lymphoid replication in the preclinical stages of disease is unknown; therefore, it is not clear whether the peripheral pathogenesis more closely resembles BSE or natural scrapie in sheep.

Effect of genetic variation in susceptibility

A small proportion of sheep with $A_{136}Q_{171}/A_{136}Q_{171}$.*PrP* genotypes do not die of infection after natural or experimental exposure to scrapie and BSE, or have very prolonged incubation periods.[26-28] The reasons for this variability in response are not clearly understood, but it can be predicted to reduce infection rates in both donor and recipient sheep in the BSE transfusion experiment. The majority of preclinical donor sheep (8 of 11) in the BSE transfusion experiment were killed at, or shortly after, the time of donation, and none showed conclusive evidence of infection, although 2 transmitted infection to their respective transfusion recipients. It is potentially significant that donors that failed to transmit infection were heterozygous at *PrP* codon 154, whereas those that did transmit infection were homozygous. Thus, variable susceptibility to infection among the donor sheep may be the result of a protective effect of codon 154 heterozygosity to oral challenge with BSE, although more data are required to confirm this association.

A novel polymorphism, resulting in a proline to leucine substitution at codon 168 of the *PrP* gene, was identified in 4 BSE transfusion recipients and 2 positive control sheep inoculated intravenously with BSE.[14] All 6 survived more than 2000 days without developing clinical signs of BSE, but on postmortem examination 4 showed PrP^{Sc} deposition in brain and lymphoid tissues. This suggests that the P168L polymorphism can protect against clinical disease but does not prevent infection by the intravenous route. This polymorphism has not been identified in the Edinburgh NPU Cheviots used as donors in the BSE experiment or in sheep with the VRQ/VRQ genotype.

Although the genetic basis of susceptibility to BSE infection in sheep and humans is not directly comparable, the variability in response to BSE found in ARQ/ARQ sheep provides a more realistic reflection of the situation with vCJD in the human population than the very uniform susceptibility of VRQ/VRQ sheep to scrapie infection. In addition, the survival of BSE-infected transfusion recipients for up to 7 years without clinical signs demonstrates that prolonged secondary incubation periods and/or a subclinical/"carrier" state are possible after transfusion in sheep. The existence of such subclinical or prolonged preclinical infection states in humans is recognized as one of the important factors influencing the probability of onward transmission, and thus the potential size of the vCJD epidemic.[29] Susceptibility to human TSEs has been linked to codon 129 of the *PrP* gene, which can encode either methionine (M) or valine (V). Until recently, all clinical cases of vCJD (including the 3 transfusion-related cases) that have been tested have been homozygous for methionine at 129 (129MM). Interestingly, the "preclinical" patient thought to have been infected by transfusion was heterozygous (129MV).[21] There is accumulating evidence to suggest that all human 129 genotypes may be susceptible to vCJD infection, with apparently greater likelihood of subclinical infection in 129MV and 129VV persons.[30-32]

Effect of blood component

The 4 transfusion-related vCJD infections occurred in patients who received transfusions of red cells that had not been leukodepleted. Leukodepletion was introduced in the United Kingdom in 1999 to control the risk of transmission of vCJD by blood transfusion because previous studies in rodents had shown that infectivity appeared to be concentrated in the buffy coat, which contains most of the blood leukocytes.[4] Subsequently, leukodepletion of blood from scrapie-infected hamsters was shown to remove up to 72% of infectivity.[33,34] In the sheep experiments, only whole blood and buffy coat were transfused because we were seeking to establish proof of principle of transmission of TSEs by blood transfusion, and assessing whether infectivity appeared to be concentrated in the buffy coat. The effect of leukodepletion was not investigated but is being addressed in a follow-up study, along with estimates of the distribution of infectivity among other blood components, including plasma, platelets, and red cells.

In our experiments, transmission rates did not appear to be significantly different in recipients receiving whole blood compared with recipients transfused with buffy coat. The number of

sheep transfused with buffy coat in [illegible] allow statistical analysis. In the [illegible] recipients were transfused with buffy [illegible] similarly to transfusion rates for [illegible] contain approximately equivalent [illegible]

We have shown that, for sheep [illegible] high transmission rates can be [illegible] particularly when donors are [illegible] period. Our results also reveal [illegible] incubation periods for subclinical [illegible] of BSE-infected blood, which is [illegible] variation in the sheep *PrP* gene. [illegible] tiers of infectivity in blood is [illegible] need for ultrasensitive methods of [illegible] It may be that, in vivo, infectivity [illegible] levels of protease-resistant PrP, but [illegible] and blood-borne infectivity in sheep [illegible] define the relationship. The results [illegible] experiments are consistent with [illegible] associated vCJD transmission in humans [illegible] sheep as an experimental model in which [illegible] with different blood products, the effects [illegible] the development of diagnostic and screening [illegible]

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Authorship

Contribution: P.H. designed the study, [illegible] postmortems on transfused sheep, [illegible] paper; A.C. performed Western blot [illegible] reviewed the report; S. coordinated [illegible] tems on donor sheep; S.M. analyzed [illegible] and reviewed the report; S.S. and I.G. [illegible] IHC results, analyzed data, and reviewed [illegible] the interpretation of IHC results and [illegible] design of the study, analyzed data, and [illegible]

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TRANSFUSION

Transfusion

What is RSS?

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REVIEWS

From mad cows to sensible blood transfusion: the risk of prion [illegible] labile blood components in the United Kingdom and in France

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ABSTRACT

Transfusion transmission of the prion, the agent of variant Creutzfeldt-Jakob [illegible] Subjects infected through food may transmit the disease through [illegible] to date by this threat are the United Kingdom (UK) and France. [illegible] the UK over the past 5 years. In France, a few individuals who developed [illegible] leading to a risk of transmission to recipients, some of whom could be [illegible] large-scale screening test, it is impossible to establish the prevalence of infection [illegible] and transfused patients. This lack of a test also prevents specific screening of [illegible] transfusion transmission essentially relies at present on deferral of "at-risk" individuals [illegible] in both white blood cells and plasma, leukoreduction is probably insufficient to [illegible] In the absence of a screening test for blood donations, recently developed [illegible] Furthermore, while the dietary spread of vCJD seems efficiently controlled, uncertainties [illegible] the spread of prions through blood transfusion and other secondary routes.

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ARTICLE TEXT

The first case known in the history of medicine as "mad-cow disease" [illegible] United Kingdom (UK),[1] where the epidemic spread widely by 2001, [illegible] by bovine spongiform encephalopathy (BSE). Most likely, the epidemic [illegible] livestock with animal food prepared from residues from slaughtering and [illegible] which died from scrapie and cattle affected by a sporadic form of [illegible] in 1988 in the UK and in 1994 in France. On March 20, 1996, the UK [illegible] BSE agent was transmissible to man. A new human pathology was [illegible] Creutzfeldt-Jakob Disease (CJD), transmitted by a variant of the [illegible]

Unlike other transfusion-transmissible agents, the prion (Proteinaceous [illegible]

and is composed purely of protein.[2,3] The "normal" prion or PrP^{C} ("proteinaceous particle") is a protein expressed on the cellular membrane by a number of tissues, but the greatest amount is found on the neurons in the brain. Sensitive to the action of proteolytic enzymes, PrP^{C} has a half-life of a few hours. Despite having an identical amino acid sequence to that of the normal form, the BSE agent is a prion of different conformation, designated by the abbreviation PrP^{Sc} (sc for scrapie) and is derived from the isoform of the normal protein by a posttranslational structural modification and conversion to a richly beta-pleated sheet.[4] The abnormal prion has a tendency to aggregate and above all a resistance (from which is derived its other abbreviation, "PrP^{res}") to proteolytic enzymes (notably proteinase K), resistance lying in the majority conformation in beta-pleated sheets.[5] The prion itself plays a role of cofactor in this conformational change. In infected subjects, PrP^{Sc} induces, on native PrP molecules, a conformation that confers on them their pathologic character, and the phenomenon of amplification is self-propagated.[6] Because it affects the accumulation of a protein present in its natural state in the body, there is no immunologic response: neither the production of antibodies nor a specific cellular response. Accumulation of abnormal prions generates vacuoles in the cerebral tissue, giving eventually a spongiform appearance and hence the title "spongiform encephalopathy."

HUMAN PRION DISORDERS

The human transmissible spongiform encephalopathies (TSEs) generally follow a long incubation period, but with subsequent rapid evolution and death. Various forms are described:

- The idiopathic disorders, principally the sporadic form of CJD (sCJD), which continues to be the commonest form. It appears predominantly in the seventh decade, with an annual incidence of 1 to 1.5 cases per million population.[7] Death follows within 6 months. This form, which is associated with a pathologic prion, has unknown etiology. It probably results from a spontaneous conformational modification of PrP to PrP^{Sc}.
- The genetic forms: familial CJD (fCJD), Gerstmann-Strausler-Scheinker syndrome, and fatal familial insomnia.
- The acquired forms were, until 1966, of human origin: kuru, found in New Guinea and linked to funeral practices, and the iatrogenic form of CJD, seen after use of contaminated neurosurgical instruments, corneal grafts, dura mater, and intramuscular injection of pituitary hormones, obtained from cadavers. Acquired disease of bovine origin, that is, variant CJD (vCJD) is seen in subjects infected with the BSE agent.[8,9] This is the only human prion disorder that has crossed the species barrier. The first series of 10 patients was described in 1996 by the UK National CJD Surveillance Unit (NCJDSU) based in Edinburgh. The disease was found chiefly in adults under 40 years of age, contrasting with the mean age for sCJD. The disease is fatal in a mean of 14 months, which is slower than the sporadic form.[10] Nuclear magnetic resonance imaging scanning shows hypersignals situated in the posterior thalamus ("pulvinar sign"). Unlike other human prion disorders, notably sCJD, in which the accumulation of abnormal prion protein affects the central nervous system with a minimal peripheral involvement, vCJD progresses with invasion, by the abnormal protein, of the central nervous system, the peripheral nervous system, and other tissues, notably lymphoid: tonsils, appendix, Peyer's patches, and thymus.[11] Tonsillar biopsy may reveal the presence of PrP^{Sc}, but a negative result does not categorically exclude the diagnosis.

GENETIC INFLUENCES

The prion protein gene is situated on the short arm of chromosome 20 and codes for 254 amino acids, with either valine (V) or methionine (M) at position 129. With two copies of the gene, an individual can be MM (39% of the normal population), MV (50%), or VV (11%). This polymorphism is fundamentally important in the development of the variant type of the disease, since there is a host susceptibility linked to the genetic type.[12,13] Homozygosity for M (MM) appears to confer susceptibility to clinical expression and to influence the incubation period of the disease: all vCJD cases, to date, in whom codon 129 typing has been performed, are MM homozygotes.[14] In one case of infection, where the individual died 5 years after an implicated blood transfusion but did not have any clinical symptoms of vCJD at the time of death, the genotype was MV. Furthermore, PrP^{Sc} has been detected in the appendix of two VV cases.[15] The MV genotype and perhaps more the VV genotype could confer a protective effect, but this remains true only until a symptomatic case of vCJD is described in a MV or VV subject. In fact, we know that individuals with all three genotypes can accumulate PrP^{Sc} in vCJD-specific tissues, but we do not know whether symptomatic cases will develop in all genotypes.

EPIDEMIOLOGY OF vCJD

To December 2007, a cumulative total of 166 cases of vCJD have been reported in the UK, which accounts for the majority of cases worldwide. Mean age of affected individuals is [illegible] with a slight male predominance. The mean duration of the symptomatic phase is [illegible] months). All tested patients were homozygous MM. The most probable origin [illegible] dietary: no risk factor of other kinds of CJD was observed.[16,17] A small number [illegible] transfusion, and some of these have been linked with a known infected donor.

In France, vCJD incidence was, as in the UK, proportional to dietary exposure to [illegible] contaminated beef into France increased regularly from 1985 to 1995, while the [illegible] decreased in the UK over the same period.[18] However, the level of exposure in France is estimated to have been 10 to 20 times lower than that of the UK, with moreover a difference between the two countries in dates of occurrence: the comparison between the number of French cases and the number of UK cases (taking account the year of the beginning of the symptomatic phase) indicates that a similar incidence in France occurred 5 years after the peak of the epidemic in the UK, where the number of recorded cases has decreased since 1999.[17] This temporal gap in the epidemic in the UK and in France is attributed to the maximal exposure of the general population in the two countries.

Between 1996 and 2007, 23 vCJD cases have been registered in France, with a median age of [illegible] 58 years) and an equal sex ratio (12 males, 11 females). The clinical and paraclinical characteristics are similar to those of the British vCJD patients. The mean duration of the symptomatic period is [illegible] months). All the analyzed cases were homozygous MM, without any risk factor other than [illegible] stays in the UK (less than 10-day periods) were mentioned in 3 patients. Four patients had lived in the UK, for long periods, between 1987 and 1996.

In other countries, vCJD cases remain exceptional. A small number of these cases have occurred outside the UK, but there remain a number which appear to have been acquired outside the UK: 3 cases each in Ireland, the Netherlands, Saudi Arabia, Spain, and Portugal and one in each of [illegible] and Japan.

Several studies have been conducted to estimate the extent of the vCJD epidemic. [illegible] British retrospective study revealed the presence of the abnormal prion [illegible] 12,674 individuals without clinical vCJD:[22] this rate appears higher than [illegible] recorded in the general population. From these results, a prevalence of [illegible] was proposed (95% confidence interval, 49-792). In the most pessimistic hypothesis [illegible] highest range of this interval), 41,250 of 60 million individuals would be infected. [illegible] the latest count of vCJD cases in the UK is more in agreement with the lower [illegible]

In France, where the level of exposure was lower than in the UK, estimations of [illegible] 60 years have been suggested in one study,[19] and 205 cases in another.[20] In 2006, a modified prediction suggested a total number of 33 cases (0-100), with 14 cases (0-30) over the 2004-11 (1-20) over the 2006-2010 period.[18] These data are compatible with the [illegible] A recent study predicted 39 (6-99) subsequent cases.[21] The worst case [illegible] years is, however, maintained in the epidemiologic estimations, in particular to estimate the [illegible] infection in the blood donor population.

Measures taken against the vCJD epidemic, with screening for the BSE agent in [illegible] animals from the food chain, and the latest epidemiologic observations suggest that a vCJD [illegible] origin is unlikely in the coming years. The unknowns now reside in other sources of [illegible] cellular vectors: blood transfusion, grafts of tissues or organs, or use of medical [illegible] contaminated with the abnormal prion. Transfusion transmission is especially feared, due to its [illegible] Nowadays, since food contamination, which was the main source of infection, [illegible] transmission by blood components taken its place? In the UK, as in France, where [illegible] eliminated from beef, they are likely to be present in the blood of asymptomatic human carriers [illegible] to the recipients of their blood donations. The fear of human-to-human transmission [illegible] interspecies contamination.

EXPERIMENTAL BASIS OF vCJD TRANSMISSION BY TRANSFUSION

Until about 1996 it was acknowledged that the CJD agent was not transmitted by transfusion [illegible] failed to show any association between the occurrence of sCJD and a past transfusion.[24-26]

higher in vCJD than in sCJD,[27] the number of infectious particles in blood and/or their distribution in individuals affected by sCJD are presumably too low to cause transmission through a blood transfusion. Thus, before the first vCJD cases, the two main circumstances of prion transmission between humans had been kuru and iatrogenic contamination by injection of growth hormones of pituitary origin. It should be noted that transmission of the kuru agent belongs to the past since the prohibition of certain rituals in New Guinea and that the exclusive use of growth hormones of recombinant origin put an end to iatrogenic transmissions through this route. Though no cases of human transmission of vCJD had yet been described, the possibility of transmission by blood transfusion remained a theoretical risk.[28-31] Unlike major transfusion-transmitted viruses observed in the past decades (hepatitis B virus, human immunodeficiency virus [HIV], hepatitis C virus), the vCJD agent did not immediately enter into the family of blood-borne agents.

In experimental models, invasion of lymphoid tissue by abnormal prion has been observed rapidly after infection, with persistence throughout the whole incubation period. It has been suggested (but not demonstrated) that the lymphoid infiltration is brought about by circulating cells, which led to the hypothesis that infected lymphocytes could transmit the prion to recipients of blood components containing lymphocytes.[32] Intracerebral injection in mice of buffy coats and plasma collected from patients with vCJD has not shown such transmissibility,[33] but these experiments only involved a small number of cases and the sensitivity of the technique may have been insufficient to detect low level of infectivity. Subsequently, transfusion transmission of prions was shown in rodents,[34] in particular in mice made susceptible to vCJD.[35,36] However, the turning point was the result of experiments aiming to show transmissibility through blood from orally infected sheep to healthy sheep:[37] it was then found that the abnormal prion was present in circulating blood and that blood could be a vector of transmission. Blood infectivity being thus demonstrated, at least in certain circumstances, French and British Health Authorities, as a precaution, considered the possibility of transmission of the vCJD agent by transfusion.

In another experiment, transfusion of healthy sheep with blood from infected sheep led to transmission rates of 17 percent for BSE and 19 percent for scrapie.[38] A more recent animal experiment was based on detection of PrP^{sc} in blood of hamsters experimentally contaminated by the scrapie agent through intraperitoneal inoculation of infected brain tissue.[39] In both cases, the infectious agent was present in circulating blood during a part of the incubation phase of the disease, and the transmission rate was shown to be quite high. However, it is important to distinguish the studies conducted with a Western blot assay detecting the amplified amyloid protein and those involving a titration of endogenous infectivity.

Finally, even before the description of the first human transfusion cases, these animal experiments had shown blood transmissibility of prions and the possibility of a short incubation period of the disease through this transmission route.

SURVEILLANCE OF TRANSFUSION RISK OF CJD IN THE UK

The first UK epidemiologic studies did not suggest transfusion as a mode of transmission of the vCJD agent, and the first descriptions of recovery of abnormal prions within the body had indicated that the blood route would be an improbable source of contamination. Subsequently, experiments into blood-borne transmission of the BSE agent in sheep and the observation of a wider distribution of PrP^{sc} in the body of subjects infected by the variant agent compared with subjects infected with sCJD led to reconsideration of this view.

In 1990, the UK, the country most exposed to the BSE risk, put in place a national surveillance system named "The National Creutzfeldt-Jakob Disease Surveillance Unit" or NCJDSU, charged with identifying and monitoring all cases of CJD.[40,41] All suspected cases were to be reported by health professionals (principally neurologists and neuropathologists) and then confirmed and categorized according to the defined diagnostic criteria. As far as transfusion is concerned, the medical history of each patient was examined and family members were interviewed, looking for history of blood donation or of receipt of transfusion. A collaborative study between the NCJDSU and the UK Blood Transfusion Services, called "TMER" (Transfusion Medicine Epidemiology Review), was set up in 1997 to examine all cases of CJD, including sCJD, fCJD, and vCJD, who had either donated or received blood in the past. On December 1, 2007, among the 166 UK cases of vCJD, 150 were old enough to have been blood donors and, among these, 31 (21%) had, at least according to their families, donated their blood at least once.[42] Records were checked and the dates and places of the donations were established. The fate of the donations was traced, including whether they were used for blood component preparation and/or for fractionated plasma products, and the fate of recipients of blood components was established. These enquiries identified donor records relating to 24 individuals who later developed vCJD; 18 of whom had donated blood that had been used to prepare components issued for hospital use. A total of 66 recipients were identified from these 18 donors; 23 of these are still alive. Blood donor records were identified for only 3 of 93 individuals who later developed sCJD and were reported to have been donors in the past, with 20 recipients identified, of whom 12 are known to be dead: 5 died within 1 year of the blood transfusion and 7 between 1 and 7 years after transfusion. Two cases with neurological sCJD at donation died and have survived 7 to 9 years after transfusion. Three cases with neurological fCJD at donation (3 donors) were associated with 11 identified recipients, of whom 3 died within 1 year after transfusion, 3, 10, and 17 years after transfusion. Three recipients not known to be dead have survived 7 to 9 years after transfusion. Among the 97 recipients thus identified, 4 have developed clinical vCJD infection and died of the disease; these all belong to the first group, those exposed to the risk of vCJD. Despite evidence that either sCJD or fCJD has been transmitted by blood transfusion, none of these recipients was informed and none were tested for evidence of infection.

THE FIRST UK CASES OF vCJD IN RECIPIENTS OF BLOOD COMPONENTS

All four transfusion-associated vCJD infections occurred in patients transfused in the UK with red blood cells (RBCs). There have been no transfusion-associated cases of sCJD or of fCJD, no case described in these two latter groups, even in retrospective lookbacks or in case-control studies. Infections have been detected in the blood in experimental animal studies, even in transgenic mice susceptible to the disease, although it is not possible to formally exclude transfusion cases that may have passed unnoticed if they possessed exceptional features and/or a particularly long incubation.

The first of the four patients infected with vCJD through blood transfusion was a case in the first group, who developed the illness in 2002 and died the next year. During surgery in 1996, he received nonleukoreduced RBCs, one of which was donated by a young donor who developed vCJD in the following year. Both donor and recipient were MM homozygous. Infection of dietary origin could be excluded in this case (as in the others), but the transfusion was the most plausible explanation for the recipient which was greater than the median for cases believed exposed through the diet. The association of this rare disease in both donor and recipient statistically was 1/3 demonstrated that these two observations of vCJD would have happened independently, if transfusion was not the cause of infection, was in the order of 1:15,000 (and rose to 1:30,000 taking into account the age of the donor). The first transfusion-associated case in world literature was reported in December 2003.[43]

The second case was an elderly recipient, who died of cardiovascular disease without having developed signs of vCJD. Asymptomatic infection with PrP^{sc} was established by post-mortem examination, with the presence of abnormal prion protein in lymphoid tissue (the spleen and the peripheral lymph nodes, tonsils or appendix), but not in the brain. This patient had been identified as "at risk" since September 1999, a nonleukoreduced RBC component had been provided from a donor who died of vCJD after donation. The PrP^{sc} isolated from the spleen had an isoform identical with that observed in the donor. The donor was an MM homozygote, but the recipient was a heterozygote (MV), which may explain the unusual nature of this case, assigned as "preclinical" or "subclinical" vCJD. Admittedly, the recipient may have developed vCJD at a later date, if survival had been longer. This second case of possible transfusion transmission of infection was reported in July 2004.[49]

The third case, reported in 2006,[42,50,51] was also one of the cohort of patients who had received nonleukoreduced RBCs and had been notified of their risk. This recipient, who had been 2 to 3 years old, had received transfusion support during a surgical intervention completed only a few months before the donor developed vCJD in 2005, 7 years after the transfusion episode, and died 2 years after. One recipient presented with vCJD in 1999 (18 months after the donation) and died the following year (2 years after blood donation). Both donor and recipient were MM homozygotes.

The fourth and last case to date was a recipient who developed vCJD 6 years after transfusion from a donor who presented with vCJD 17 months after donation. This donor was the same donor as that for the third case. The recipient, genotype MM, died 1 year after presentation.[52]

These cases reported in professional journals (and subsequently in government reports) have established transfusion-associated vCJD moving progressively from "theoretical risk" to "demonstrated" and then finally "demonstrated." There are a number of unknowns in the various estimates of risk per donation, but the combination of the low prevalence of vCJD in the general population (the estimation for an individual unit varies between 1:15,000 and 1:30,000 in the UK)[53] and of the high prevalence of infection in the small group of recipients who have been rendered at risk (and it should be borne in mind that almost all these at-risk recipients have died without surviving long enough to develop an overt disease like vCJD or be tested for the presence of infection) makes highly probable a transfusion risk higher than 1:2,000. All these cases reinforces the theory that the blood of a donor in the asymptomatic phase of the disease is infective for recipients. This evidence of the transfusion transmissibility of vCJD has largely justified preventative measures previously applied in the UK and in France.

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In fact, despite the small number of reported transfusion cases, many observations have been proposed or are already known:

1. The possibility of a relatively short incubation period with a transfusion source: 6½ years between the transfusion and the first clinical signs in Case 1, 6 years in Case 3, 8½ years in Case 4. This short incubation period demonstrates the efficacy of the transfusion route. It might suggest a particular pathogenic character of the abnormal prion circulating in the blood and transmitted by this route, even if it is established that intraspecies transmission is usually accompanied by a shorter incubation than interspecies transmission. Indeed, the shortest incubation period has been observed in kuru, in the iatrogenic form after injection of growth hormone,[53] and in transfusion-associated vCJD.[54]
2. The rate of transmission in the population of at-risk recipients is high, even though it is not inevitable in the relatively short follow-up period.[55] A review of the UK's TMER study published in 2006 gave an indication of the transfusion risk of vCJD and of the incubation period of the first observed cases:[42] among the 66 blood component recipients transfused from donors who later developed vCJD, 37 died within the first 5 years posttransfusion with a cause of death linked to the existing illness. Apart from the one case shown to have evidence of infection, none of the other deceased recipients were tested for evidence of infection because their deaths predated the information that their donors had developed vCJD. Furthermore, no postmortem tissue was available for retrospective testing.

Among the 29 who survived over 5 years, 20 are still alive and have no signs of vCJD, and 9 are now deceased. Among these 9, 6 died of pathology not linked to vCJD (although only 1 of these had a postmortem to look specifically for infection, which was demonstrated) and 3 developed (and died from) vCJD.

3. The influence of codon 129 genotype is not refuted in the context of the transfusion route: the sole recipient known to be infected but asymptomatic was a heterozygote (MV), although it should be noted that the observation period was the shortest of the series of infected recipients, since this recipient died 5 years after transfusion.
4. All the infected recipients had received nonleukoreduced RBCs between 1996 and 1999. Routine leukoreduction was introduced in the UK by October 1999.
5. The four recipients who developed evidence of infection had been transfused respectively with components from 5, approximately 8-10 (figure uncertain), 56, and 23 blood donors. [Correction added after online publication 2-Jan-2009: Number of donors has been updated.]
6. In the UK and France, no case of vCJD has been reported in recipients of fractionated plasma products. As indicated in the title of this article, we have limited our review to labile blood components, aware of the additional procedures that contribute to the safety of plasma products with respect to prions.

SURVEILLANCE OF TRANSFUSION RISK IN FRANCE: FIRST CASES OF vCJD WITH PREVIOUS BLOOD DONATIONS AND FIRST MEASURES TAKEN WITH REGARD TO THE RECIPIENTS

Although epidemiologic investigations conducted in France have not revealed previous blood transfusions during the "risk period" for vCJD (one case had received a blood transfusion, but in 1971, before the epidemic), some patients had been blood donors, as would be predicted, in the same period. In 1992, a national surveillance network for cases of CJD was set up in France, coordinated by Inserm Unit U708 and including representatives of various medical specialities and the health services: neurologists, neuropathologists, reference laboratories, and the "Institut de Veille Sanitaire" (InVS). The aim of this network was to collect and investigate reports of suspected cases of CJD, follow their progress, classify the type (sporadic, familial, iatrogenic) and the degree of probability (distinguishing confirmed cases from probable cases), and establish epidemiologic characteristics. In cases with previous history of blood donation, InVS was charged with informing the French Blood Service ("Etablissement Français du Sang") so that a transfusion investigation could be started. It appeared that three of the French vCJD cases, who had developed the disease in 2004, had a history of blood donation.

The first case (eighth in the series, reported in February 2004) was a 32-year-old female who donated blood between 1993 and 2003. The components prepared from these donations were 13 concentrated RBCs (of which 10 were leukoreduced) and one platelet (PLT) concentrate. Fourteen recipients, of whom 10 were still alive, were traced. Ten plasma donations were used for fractionated plasma products.

The second case (ninth in the series, reported in April 2004) was a 52-year-old man who had donated blood since 1984, chiefly between 1996 and 2002. No investigations were carried out into donations which preceded the vCJD epidemic. The blood components were 5 concentrated RBC units (all leukoreduced) and 5 [illegible] leukoreduced). For donations made after 1994, 7 recipients were traced, of whom 3 were alive; donations were used for fractionation.

The third case (13th in the series, reported in October 2004) was a 46-year-old man who [illegible] 1991 and 2004. The components were one fresh-frozen plasma (FFP) and 15 concentrates (all of them leukoreduced). All 16 recipients were identified, of which 6 were alive.

In total, these 3 donors account for 42 recipients of RBCs or PLTs, of whom 16 were alive at the investigation: 2 of these, transfused before 1984, were not informed, but 14 were notified of their transfusions between 1991 and 2004. To date, none has presented with symptoms of vCJD. Two recipients were tested for evidence of infection, because all died several years before the diagnosis of donor. There were clearly more recipients of fractionated plasma products prepared from plasma of affected donors. Two of the donors had given plasma destined for fractionation in the period [illegible] donations in one case, 12 in the other). These 2 donors accounted for around 59,000 recipients: treatment of chronic disorders (hemophilia, immunodeficiency), the rest for occasional treatment (e.g., immunoglobulins).

In response to the first three cases of blood donors who later developed vCJD, the following measures into place in France:

- Immediate recall of in-date fractionated plasma products and labile blood components [illegible] donors. When the illness was discovered in the donor, blood products had almost always been transfused, but this strategy allowed the following actions.
- Information to the prescribers of the labile blood components implicated in the investigation.
- Direct and personal information to the recipients of blood components (except those [illegible] epidemic); exclusion of all recipients as donors of organs, tissues, and cells [illegible] blood donation because of their history of transfusion); and finally, putting in place long-term follow-up.
- A decision to not inform individual recipients of fractionated plasma products, except those who received Factor (F)VIII or F IX produced from the affected donations.
- Information aimed at the general population and at health professionals about the risk of transmission of vCJD.

The information given to the blood transfusion recipients by their doctor proved reminiscent of [illegible] more than 20 years previously, to the first blood donors to be found "LAV positive," who were told of the large number of uncertainties at the time about the prognosis of infection by the agent. Those who supported not informing recipients of the risk of prion transmission through blood transfusion put forward the following arguments: it is not possible to quantify the absolute risk, because of a number of unknowns and the absence of a diagnostic test; the existence of preventive measures applied in recent years [illegible] to labile blood components; the major psychological harm resulting for such information, which can [illegible] major anxiety; absence of any diagnostic or prognostic tests (except for codon 129 status) [illegible] prophylaxis or treatment. On November 4, 2004, the National Ethical Consultative Committee [illegible] confirmed its position expressed in 1997: to not worry without benefit, notably where no preventive [illegible] available, and to take into account the risk of excluding a patient from health care in the name of the precautionary principle. Finally, the CCNE insisted on the need for complete traceability of donations and donors who had subsequently developed vCJD.

Those in favor of informing recipients of the risk pointed out the need to inform them that they [illegible] donate (in principle, because they had been transfused, the subjects were already excluded permanently from blood donation), but also that the patients had "the right to know," imposed by French and European law which puts an obligation on the doctor to alert the patient to all "newly identified risks," even when the individual risk is not quantifiable and there is no available diagnostic procedure and no means of prevention. Another factor favoring informing recipients is to reduce the risk of secondary cases to [illegible] dentists, and other patients. The French circular number 138 of March 14, 2001, [illegible] principles of the risks of transmission of "nonconventional transmissible agents" during medical procedures and had classified the recipients of labile blood components in the category of patients at risk of contamination by the vCJD agent. For all these reasons, in France, it was ultimately decided to inform patients at high risk of prion infection.

PRECAUTIONARY MEASURES FOR DONORS AND LABILE BLOOD COMPONENTS IN FRANCE

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Since the removal of infected beef products from the food chain, a public health measure taken to protect the general population, precautionary measures to reduce the risk of transfusion transmission of prions were implemented in the UK and France in line with advances in epidemiologic knowledge. Some were put in place before the emergence of the first case of transfusion-associated vCJD, primarily to reduce the risk of transmission of other forms of CJD and in particular the iatrogenic forms. The first case of transfusion transmission of vCJD provoked the health authorities in the UK and France to take new and complementary risk reduction measures. Along with the exclusion of at-risk donors, the introduction of leukoreduction has contributed to the reduction of the infectious load in prion transmission by blood[56] (it has been shown that this could reduce the infectivity of whole blood by almost 50%[57]). Despite this, as the cases of vCJD transmission by blood transfusion observed in the UK were all due to nonleukoreduced blood components, it could be concluded that the decrease is by definition accounted for by leukoreduced blood components, and above all that it has been established that the white blood cell (WBC) layer does not contain all the infectivity: an equal amount of infectivity exists, we now know, in plasma. Leukoreduction therefore appears a necessary measure, but certainly not sufficient.

Table 1 lists, in chronological order, the precautionary measures, specific or nonspecific, put in place in the UK[58,59] against the risk of transfusion transmission of vCJD. In France, the precautionary measures followed the same pattern in a number of complementary actions. The circular of September 23, 2005,[60] concerning the reports of the first probable British cases of transfusion transmission of vCJD and the first case of a French donor who developed the illness, raised the issue of secondary transmission by transfusion of labile blood components or by use of surgical instruments or endoscopes on patients who had received transfusions of blood components originating from donors who later developed vCJD. The successive measures instituted in France and including those taken for the other forms of CJD before the emergence of vCJD, are shown in Table 2.

TABLE 1. Preventive measures in the UK against the transfusion risk of vCJD

1997	Recall and discard of labile blood components and of plasma derivatives obtained from donors who later developed vCJD
1998	Importation of plasma destined for fractionation from non-UK sources.
1999	Leukoreduction of all labile blood products.
2000	Importation of FFP for recipients born after January 1, 1996.
2004	Permanent donor deferral in case of transfusion after January 1, 1980.
2005	Importation of FFP for recipients age less than 16 years.
	Permanent donor deferral in case of transfusion anywhere in the world after January 1, 1980.
	Permanent deferral and notification of donors whose donations have been transfused to recipients who later developed vCJD.
	Progressive replacement of PLT pools with apheresis (single-donor) PLTs. Apheresis PLTs recommended for children age less than 16 years.

TABLE 2. Preventive measures in France against the transfusion risk of vCJD

1992	Permanent donor deferral in case of treatment by injection of growth hormones of pituitary origin.
1996	Permanent donor deferral in case of history of neurodegenerative disease.
	Recall and discard of labile blood components and batches of plasma products containing plasma from donors who later developed sCJD, fCJD, or iatrogenic CJD; having a history of fCJD; or having been treated with hormones of pituitary origin.
	Permanent donor deferral in case of history of neurosurgery.
1997	Tracing of recipients of labile blood components collected from donors who later developed CJD.
	Permanent donor deferral in case of transfusion of graft.
	Recall and discard of labile blood components and plasma products obtained from donors who later developed vCJD.
1998	Leukoreduction of cellular blood products for a residual level <1 × 10^6/unit.
2000	Permanent deferral of donors who lived in UK for 1 year or more between 1980 and 1996.
2001	Leukoreduction of all plasma (FFP or plasma destined for fractionation) to a residual level <1 × 10^6/unit.
	Residual WBC level <1 × 10^6/unit for plasma not destined for fractionation.
	Reduction of volume of plasma in PLT components through use of PLT additive solution, potentially reducing an infectious load.

One difficulty with the current situation is that individuals incubating vCJD [illegible] they are at risk and may be donating their blood. This is relevant as they [illegible] relatively young and could donate their blood several times per year. [illegible] only specific preventive measure against prion contamination of blood transfusion [illegible] blood test for qualification of donors, or a general measure which could [illegible] for prion filters), or both.

In the prevention of any transfusion risk, an equilibrium between the [illegible] blood components is necessary. Being the most exposed country, the UK has [illegible] transmission by blood transfusion, such as the importation of all plasma [illegible] the exclusion of numerous blood donors,[62] these measures were [illegible]

Transfusion measures taken in other countries are essentially based [illegible] have stayed in an "endemic" area. For example, the Canadian authorities [illegible] measures to exclude from donation individuals who became at risk [illegible] vCJD epidemic: in 1999, all people who had spent a cumulative period [illegible] excluded from donation; in 2000, it was the same criteria for [illegible] France or the UK was reduced to 3 months; and that of a stay abroad [illegible] period of 5 years.[65,66]

After a case of vCJD in an individual who visited the UK for less than [illegible] 2001 an illness that led to his death in 2004, Japan also took precautionary [illegible] considering that the patient had become infected in the UK, even though [illegible] have been identified with BSE. Having already excluded donors who had stayed [illegible] Japanese health authorities took the decision to exclude all individuals who had [illegible] country between 1980 and 1996. One can see that prion infection and [illegible] two common points: they cross all frontiers and spread in an unforeseen manner.

THE VARIABLES OF RISK OF TRANSFUSION TRANSMISSION OF [illegible] COMPONENTS

At this stage of medical knowledge, it is clear that all the elements of [illegible] are not clarified. Certain elements are however identifiable:

1. The number of labile blood components received by the patient and [illegible] to the dates of the epidemic and to the application of precautionary [illegible] leukoreduction, etc.).
2. The prevalence of infection in blood donors: a great uncertainty [illegible] upon that in the general population who were exposed throughout [illegible] taking into account the higher end of the estimate of between [illegible] French population and imagining these 300 cases among the [illegible] be blood donors (18-65 years) and eligible to be donors, and [illegible] the whole incubation period, results in a prevalence of 1 in 1,2[illegible] individuals per 1 million donors, which is close to 1 infected donor [illegible] In a recent reevaluation, the number of expected vCJD cases in France [illegible] 100 cases instead of 300), leading to an estimate of 1 donor infected [illegible] calculation in the UK gives a prevalence of 1 in 10,000 donors, [illegible] available in the UK with results from the National Anonymous Survey [illegible] progress.
3. Infectivity of a labile blood component with regard to prions is still [illegible] an "infectious dose," defined as the minimal dose capable of transmitting [illegible] the mode of contamination given. At present, the infection of a unit of [illegible]
   - The stage of infection in the donor: the level of circulating prion and [illegible] increases with the duration of the incubation period.[68] This might [illegible]

the infected subject becomes infective for the recipient of the blood: an infected donor, donating during the early part of the incubation period, may not be infectious to a recipient. According to animal studies, blood infectivity can be demonstrated at least at the start of the second half of the incubation period and perhaps also earlier (the infectivity of blood precedes the presence of pathological prion in the brain and the organs).[66] Even though experiments suggest that infectivity will be absent or minimal during the first third of the incubation period, caution dictates, in the current state of knowledge, that a labile blood component originating from a donor in the incubation period contains at least one infectious dose.[69] As many years have passed since the peak of the dietary epidemic, infected individuals are no longer in the initial stages of infection. The paradox could be that even though the number of infections is no longer increasing, the number of infectious subjects could still increase over time.

- Second, the efficacy of leukoreduction for cellular components and for plasma: leukoreduction of hamster blood contaminated with a scrapie prion removed only a little less than half (42%) of the infectivity present, because the infectivity divides almost equally between the WBCs and the plasma.[67,70,71] Leukoreduction may therefore be less effective than originally calculated. As demonstrated in studies based on experimentally infected rodent blood, total blood infectivity will be, during the asymptomatic phase, from 20-30 IU per mL,[35] and the distribution in the compartments of blood is in the order of 30 percent in the buffy coat and 50 percent in the plasma.[71] The presence of RBC and PLT infectivity has not been established in a formal manner: it seems at any rate to be little or none.[72,73] Thus, after the implementation of leukoreduction of labile blood components (which must have a residual WBC count of $<1 \times 10^6$/unit), the infectivity of RBC or PLT components is dependent on the amount of residual plasma. Use of optimal additive solutions for cellular components helps to reduce the quantity of plasma and therefore the infectious dose in the case of an infected blood component.

4. Recipient methionine homozygosity at codon 129 has an impact on the risk of developing illness, with perhaps a hierarchy of risk, moving in descending order from MM homozygotes to MV heterozygotes to VV homozygotes. Furthermore, nonhomozygosity for MM does not appear to confer absolute protection from infection, as indicated by the second UK recipient case (an MV heterozygote, nonetheless infected through the transfusion route) and in experimental animals.[13] What is certain is that the clinical outcome of transfusion transmission appears to be greatest for MM homozygotes, since they alone of the "exposed" population at risk have developed the disease.
5. Finally, the length of the incubation period, an essential factor and of which much is currently unknown and to which must be added two important parameters: the age of the recipient and the posttransfusion survival, which is heavily influenced by deaths due to the underlying illness in the initial years after the blood transfusion.

PRION FILTERS

Specific prion reduction filters applicable for certain labile blood components have been undergoing validation. The first donations processed with these prion filters demonstrated their capacity to reduce spiked infectivity of blood by three logs, which would without a doubt make a significant contribution to reducing transfusion risk.[74] These filters have been produced by two companies with a view to use for RBC preparations: application to PLT preparations and to plasma await further work. The validation work has been carried out on the Pall leukotrap affinity prion reduction filter, integrated in the filter CompoSafe Pr Fresenius,[75-77] and the TSE affinity ligand of the pathogen removal and diagnostic technologies, integrated in the P-Capt MC (MC for Macopharma) filter.[78] Changes were made to the Pall filter after the initial validation, which affected performance and led to its withdrawal. A new combined leukoreduction and prion removal filter from the same manufacturer is now under development. These affinity filters are assumed to remove all detectable traces of infection in a contaminated unit and to reduce infectivity by transfusion. This capacity has been demonstrated by a study based on inoculation, in hamsters, of leukoreduced whole blood taken from animals infected by a TSE. When the blood was treated with passage over a filter, no hamster became infected. When the blood was not filtered, some hamsters developed illness associated with the presence of prions in tissues.[79] Nevertheless, although the potential of these filters has been demonstrated by experimental infectivity transmissions in animal models, their efficacy in the prevention of human transfusion transmission remains to be validated.[67] Indeed, the amount and form of prion circulating in different human blood components may differ from that in a [illegible] manner, in particular from brain extracts. These artificial situations cannot [illegible] quantitative characteristics of the human prionemia. The most important [illegible] are those that are formed of 14 to 28 molecules,[81] but the size of circulating [illegible] unknown. Furthermore, the consequences of using prion reduction filters on blood components (on the maintenance of PLT function) and on plasma proteins is totally unknown.[82] One [illegible] the risk of neoantigenicity and induction of inhibitors.

A MUCH-AWAITED DIAGNOSTIC TEST

Lacking nucleic acid and not provoking any immune response by the infected host, the prion cannot be detected by molecular or serologic methods usually used in viral diagnosis. Research for such markers has, up to now, reached a dead end.[83-85]

In asymptomatic or symptomatic infection, the most useful diagnostic test will be [illegible] pathologic prion in the blood. However, the form that the prion takes in the blood [illegible] central nervous system. PrP^{sc} has an aggregated form in the brain and a [illegible] that difference could influence the effectiveness of diagnostic tests, the majority [illegible] capacity to detect the cerebral form. Furthermore, the pathologic form only [illegible] prions, but it is this pathologic form that the test must detect. Most of the [illegible] depend on the physicochemical differences in the two forms of prion, the [illegible] on the resistance of the pathological form to proteinase K.[11,86-90]

A large number of unknowns relating to the transmissibility, [illegible] no doubt be resolved when one or several diagnostic tests, having the [illegible] specificity, and reproducibility, become available and usable on a large scale.[91,92] [illegible] being made to develop such tools, which could be used in the screening of blood donations, reduce even further the risk of transfusion transmission of prions. These tests should meet the criteria:[93,94]

1. A very high sensitivity, to detect an infectious load that must be very low in asymptomatic [illegible] a low level of PrP^{sc} in circulating blood is likely to be infectious for a number of [illegible]
2. High specificity is essential, since the normal protein is present in the [illegible] results could have disastrous consequences, in terms of notifying individuals who [illegible] concluded "positive," not to mention the unjustified deferral of a large number of [illegible] infections, every reactive result obtained through blood donation screening [illegible] confirmatory test to separate true-positive results from false-positive results. [illegible] true confirmatory test will be available, whether the solution for prions will [illegible] simultaneously, or if one will be used for "confirmation" of a positive result. [illegible] specificity, it has been calculated that, if a diagnostic test [illegible] specificity was applied to the screening of blood donations in a population [illegible] in 10,000 (which is the estimate for donors in the UK), 99 individuals would [illegible] phase of the infection and would correspond to "true positives" for 1 million [illegible] donors would give a false-positive result. On the other hand, there would [illegible] 1 million tests.[96] In France, where an estimate of prevalence [illegible] carriers of the variant would be detected, but the number of false-positive [illegible] the UK: 10,000 per 1 million donors, who would not be allowed to give [illegible] informed of their biologic status.
3. Finally, these tests will have to be reproducible, usable on a large scale, [illegible] time scale that is compatible with the shelf life of PLT components, or [illegible] obtained) for nucleic acid testing in transfusion.

The lack of a test with the above-mentioned characteristics has [illegible] from blood donation all those who are carriers of vCJD and the necessity of basing the [illegible] blood donations on nonspecific or partially effective measures such as existence of a [illegible] leukoreduction of blood donations, and so forth; the impossibility of detecting [illegible] risk recipients; the difficulty in collecting data about the mean duration of the [illegible]

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and, further to all this, the impossibility of rehabilitating donors excluded because of a stay in the UK during the affected years (all the more because, among the cases of vCJD identified in France, such a history has been found only once and it becomes a paradox to exclude donors on the pretext of a visit to the UK when almost all the French patients who had the illness were infected in their own country). It would be necessary, furthermore, to take care that the positive effect of such a "rehabilitation" for some donors was not offset by a negative effect, by announcing, in the media, the use of a specific transfusion screening test. This could raise concern in the donor population, of learning, through going blood, that they carry the infectious agent of an illness for which there is no preventive or curative treatment.

While awaiting validation, and predicting their potential use in detecting donors who are infected by vCJD, the first tests could be usefully applied in studies of sample repositories, to determine the spread of the epidemic in the overall population and in the transfused population. Assessment of the prevalence of vCJD in donors and recipients of blood, as well as its transmissibility through plasma products, could be carried out via anonymous plasma samples of infected donors and recipients. This is one of the possibilities provided by the repository presently undertaken on a European scale, called "BOTTIA" (Blood and Organ Transmissible Infectious Agents).[97] Indeed, for obvious ethical reasons, one cannot use imperfectly validated tests on nonanonymous samples.

Meanwhile, the absence of a diagnostic test and strong uncertainties about a transfusion epidemic of vCJD requires maintenance of the preventive measures established by the UK, France, and other countries. If a specific test is used in transfusion in the future, there will be the opportunity to consider relaxation of these measures.

UNCERTAINTIES

An illness whose pathogenicity is not well known, with an uncertain prevalence of the infectious agent in at-risk groups and in the general population, the absence of a screening test, infectiousness and duration of incubation poorly defined, and the absence of any therapy, make up the elements that influence the transfusion risk of vCJD and handicap its prevention. Many questions have no answers, and the order in which we enumerate them probably does not correspond to the sequence in which solutions will be found:

1. After the end of the UK dietary epidemic and after the peak of the vCJD epidemic in 1999, will there be a second peak of transfusion origin? Up to now, the epidemic has remained relatively limited: approximately 600 cases worldwide, of which three-quarters have been in the UK. The initial pessimistic hypotheses on future number of cases have been revised downward. Furthermore, the peaks that followed the initial peak of CJD cases linked to injection of contaminated growth hormone were smaller and smaller, as if patients of other genotypes were less susceptible to infection and/or to the development of clinical illness. It is not known if the same will happen with vCJD, but the hypothesis of a secondary transfusion epidemic, with amplification of the phenomenon through asymptomatic carriers of the prion, cannot be excluded. Nevertheless, it is now 14 years since the first cases of vCJD occurred, and no evidence of clinical cases in heterozygotes has appeared, in contrast to observations in the growth hormone epidemic. Finally, intraspecies transmission of prions induces, compared to interspecies transmission, a shorter incubation period and increased effectiveness of transmission. This could cause a larger outbreak of infection through transfusion than through contamination by food.
2. What is the prevalence of infection in the general population of the UK and in France, and how many potentially infected donors are there? The results of a retrospective British study on the prevalence of vCJD in surgical tissues from appendectomies and tonsillectomies pointed in the direction of a much higher prevalence of asymptomatic carriers than was implied by the known number of symptomatic cases. Furthermore, since, in the British MV transfused recipient carrying the variant, PrP^{res} was only detectable in the spleen and the cervical lymph nodes, and not in the appendix or the tonsils, this retrospective epidemiologic study based on detection of the pathologic prion in the appendix could have underestimated the size of the epidemic in the general population.
3. What are the kinetics of the appearance of circulating prion during the incubation phase? For estimation of the transfusion risk, the working hypothesis is that of blood infectivity and thus potential transmissibility throughout this phase, but the prion level in circulating blood may be too low, in the first months or first years of infection, to transmit infection by transfusion.
4. What is the effect of the current precautionary measures in transfusion, especially leukoreduction? The margin of safety that this measure gives is unknown. Has a reduction in infectivity prevented, or will it prevent, some transmissions by blood components? Up to now, the most feared contradiction would be the appearance of vCJD in a recipient transfused solely with leukoreduced components. Such a finding has not yet been reported.

5. Do non-MM subjects (that is, 60% of the general population) [illegible] might they develop it after a longer period of incubation? This latter hypothesis would [illegible] the epidemic, which might, furthermore, be partially masked by other causes of [illegible] situations, infected asymptomatic subjects would not be [illegible] recipients could become symptomatic if they have the MM status. Such a situation [illegible] epidemiologically disastrous: an MV or VV infected donor [illegible] and not become ill him- or herself, but the recipients would develop illness [illegible] circumstances have been observed in viral transfusion transmissions, notably [illegible] infected recipient could develop symptomatic illness several years before [illegible] donors who are carriers of vCJD but do not develop the illness [illegible] 129 would not be identifiable without a specific diagnostic test, except by [illegible] their common donor status in two (or more) recipients infected by [illegible] because of a nonprotecting genotype. Such studies would [illegible] regular, infected donor and of interrupting a chain of transmission [illegible] extent the deferral of transfused patients from giving blood [illegible] "contamination cycle" between the donor population and [illegible] this precaution has probably avoided several transfusion [illegible] based on a mathematical model, has concluded that the effect [illegible] majority of donors were infected from dietary sources and [illegible] excluded from blood donation.[99]
6. How many donors and recipients will develop vCJD during the next few years [illegible] transfusion lookbacks and investigations? In the TMGR study, among recipients [illegible] infected donors, the proportion of recipients who developed the [illegible] period of time (less than a decade), taking into account that the [illegible] genetic status of codon 129.
7. Will the threat of transfusion transmission of prions be limited solely to the [illegible] in other countries such as Spain and Saudi Arabia, of cases of vCJD [illegible] that the problem has now taken on an international dimension, [illegible] transfusion safety.

CONCLUSIONS

The possibility of a blood component recipient developing vCJD [illegible] regular donor developing it after the same amount of time, and that [illegible] with the help of a transfusion traceability almost as prolonged as the [illegible] dietary epidemic is now a problem of chronic asymptomatic carriers [illegible] infection via medical devices used in surgery or in [illegible] of epidemic.

An essential notion is that of protection provided by [illegible] scenario, where cases of vCJD would show up in recipients [illegible] blood components and thus infected by the residual [illegible] the screening of blood donations, would be to have recourse [illegible] and harmlessness is resolved, or to only use washed [illegible] concerns[101]—even if a partial reduction of prion level [illegible] infected recipients and/or to induce a longer incubation period [illegible] proportional to the original contaminating inoculum).[102]

Many professionals in the field of transfusion infectious [illegible] acknowledging that demonstration of their clinical efficacy remains difficult for [illegible] and which are dominated by the absence of a diagnostic test usable on a large [illegible] these filters is problematic and leads to as many questions as not using them. [illegible] test that would be applicable for blood donations will raise a no less difficult question: [illegible] test?

Procedures for the inactivation of infectious agents in labile blood components [illegible] since they are aimed at the nucleic acids of these agents, they will not be effective [illegible]

the prion could become the ultimate transfusion-transmissible agent, while the risk connected to viruses, bacteria and parasites, known or emerging, would be controlled by pathogen inactivation.

If transfusion transmission of vCJD is a certainty from now on, benefits of transfusion obviously remain immeasurable compared to this risk. One must put in perspective the number of lives saved every day by transfusion and the number of cases of transfused vCJD counted on a worldwide scale. One also must compare this risk, which mainly concerns two European countries, with the infectious risks faced by transfused patients in parts of the globe where the means are so limited that safety is not always assured even for major blood-borne agents.

Never before have so many measures been taken in transfusion to counteract a risk that is numerically so low, some taken even before the first case of vCJD by blood transfusion had been reported. The precautionary principle has not just gone into the law; it has also penetrated the senses.

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bjh review

An update on the assessment and management of the transmission of variant Creutzfeldt-Jakob disease by blood and plasma products

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Summary

There have been four highly probable instances of variant Creutzfeldt-Jakob disease (vCJD) transmission by non-leucocyte depleted red cell concentrates and it is now clear that the infectious agent is transmissible by blood components. To date there in no reported evidence that the infectious agent has been transmitted by fractionated plasma products, e.g. factor VIII concentrate. This review outlines current and potential risk management strategies including donor deferral criteria, the potential for donor screening, blood component processing and prion reduction filters, plasma product manufacture and the difficulties in identification and notification of those considered 'at risk of vCJD for public health purposes'.

Keywords: Creutzfeldt-Jakob disease, blood, plasma products.

This review offers an update on our recent assessment and management of the risk of transmission of variant Creutzfeldt-Jakob disease (vCJD) by blood components and plasma products (Ludlam & Turner, 2005). As that review surveyed perceptions on the nature of the prion agent, the spectrum of prion diseases in animals and man, and the range of animal studies relating to pathogenicity and infectivity (much of which still represents the current level of knowledge), these topics are not reviewed again here, other than where significant new relevant studies have been published. This current review focuses on the state of the art in relation to the safety of blood components and plasma products, which has also been reviewed elsewhere (Farrugia *et al*, 2005; Dolan, 2006; Ironside, 2006 and Clarke *et al*, 2007).

To date, a total of 203 probable, or definite, cases of vCJD have been reported worldwide, of which 166 have arisen in the UK, 23 in France, four in Eire and Spain, three in the USA, and one in each of Holland, Portugal, Italy, Saudi Arabia, Japan and Canada (http://www.cjd.ed.ac.uk/vcjdworld.htm).

Of these, two of the [illegible] and Japan are thought to have [illegible] third US case is thought to have [illegible] The other cases are thought to [illegible] countries of origin either during [illegible] or exported animals or animal [illegible] vCJD appears to have reached a [illegible] has waned such that in 2007 in [illegible] though the frequency of new c[illegible] France and Spain. All clinically [illegible] have been methionine homozygo[illegible] protein gene (*PRNP*). Mathematic[illegible] current incidence of vCJD might [illegible] estimate of 70 further cases [illegible] (Clarke & Ghani, 2005). The [illegible] estimate, however, if individuals [illegible] are also capable of being infectio[illegible] sions occur from asymptomatic [illegible]

Two observations give pause for [illegible] median age of onset of clinica[illegible] altered over the past 10 years as [illegible] individuals were exposed to infec[illegible] of time. The best estimates [illegible] related exposures [illegible] second in the early [illegible] appendices (Hilton [illegible] showed evidence [illegible] maximum likelihood estimat[illegible] discrepancy between the estim[illegible] clinical incidence [illegible] around 85% of [illegible] pre- or sub-clinical infection. Cl[illegible] consistent with [illegible] studies in patients who [illegible] suggest that individuals [illegible] homozygous at codon 129 [illegible] and a lower incidence of dev[illegible] those who are codon 129 [illegible] observations give rise to concern [illegible] cohort of individuals, maybe a n[illegible] population in the UK may have [illegible]

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and so at risk of transmitting the disease through blood and tissue products or surgical and medical instrumentation, despite being asymptomatic themselves.

As there is no currently accepted blood test that reliably identifies vCJD infected individuals (see below), further studies have been carried out to try to refine the estimate of the prevalence of sub-clinical disease. The National Anonymised Tonsil Archive aims to test 100 000 tonsil samples. Currently, there have been no confirmed positive samples out of 43 000 tested (http://www.hpa.org.uk/infections/topics_az/cjd/tonsil_archive.htm). However there are reservations around the interpretation of these data, given that the sensitivity of the assays in detecting subclinical vCJD is uncertain, the frequency of involvement of the tonsil as a site of peripheral infection is unknown, and a large proportion of the study population are too young to have been exposed to dietary bovine spongiform encephalopathy (BSE). The Spongiform Encephalopathy Advisory Committee (SEAC) has therefore not felt it appropriate to amend the current prevalence estimates within the UK at present (http://www.seac.gov.uk).

Infectivity in the peripheral blood

Infectivity remains undetectable in the peripheral blood of patients with vCJD despite the fact that clinical transmission has clearly occurred. This apparent contradiction is probably explained by the presence of a species barrier between man and mice and the limited volumes of blood that can be inoculated in these models.

Studies in hamsters infected with the 263K strain of scrapie have yielded similar results to those in the Fukuoka-1 GSS strain in mice (Brown *et al*, 1998; Ludlam & Turner, 2005), with a point estimate of 1·6 infectious doses (ID)/ml of whole blood of which the plasma (50%) was associated with the leucocytes and most of the remainder in the plasma (Gregori *et al*, 2004). Further studies in this model suggest that the majority of cell-associated infectivity is only loosely bound and can be washed off and therefore that the plasma form of infectivity probably predominates. Further studies in mice suggest that the level of infectivity is similar in vCJD-infected animals (Cervenakova *et al*, 2003). Studies in sheep naturally infected with scrapie, or experimentally infected with BSE, suggest a transmission frequency of up to 50% from blood taken during the preclinical or clinical phase of disease and transfused into recipients from a scrapie-free flock (Hunter *et al*, 2002). BSE has also been transmitted through buffy coat to the primate *Macaca fascicularis* (Houston *et al*, 2002).

Variant CJD transmission by blood transfusion

Within the UK, the Transfusion Medicine Epidemiology Review (TMER) has proved an effective system for collating evidence of possible transmission of vCJD by blood components (Llewelyn *et al*, 2004). The UK CJD Surveillance Unit in Edinburgh shares information about new cases of vCJD with the Blood Transfusion Services, which search their databases to ascertain whether these patients have been blood donors in the past. In this event attempts are made to identify the fate of the blood components (http://www.cjd.ed.ac.uk/TMER) and trace, notify and monitor living recipients. The 'reverse' arm of the TMER study attempts to identify which individuals who develop vCJD have received blood transfusions and to identify the donors.

Eighteen patients with vCJD have, or had previously, been blood donors, from whom a total of 66 recipients have been identified, 26 of whom are still alive. Of those who have died, four cases of transmission of vCJD prions have been identified (see below). Many of these patients however will have died of their underlying conditions within 5 years of the implicated transfusion and will not have had time to show clinical evidence of vCJD if infected.

The first symptomatic case of vCJD disease associated with blood transfusion was identified in December 2003. This individual developed vCJD 6·5 years after transfusion of red cells donated by an individual who developed symptoms of vCJD 3·5 years after donation (Llewelyn *et al*, 2004).

A second case of transmission was identified a few months later in a recipient of red cells from a donor who developed symptoms of vCJD 18 months after donation. This patient died from causes unrelated to vCJD 5 years after transfusion. Postmortem investigations found abnormal prion protein accumulation in the spleen and a cervical lymph node, but not in the brain, and no pathological features of vCJD were found (Peden *et al*, 2004).

A third patient developed symptoms of vCJD 6 years and died 8·7 years after receiving a transfusion of red blood cells from a donor who developed vCJD about 20 months after this blood was donated (Health Protection Agency 2006).

The fourth case of transmission developed symptoms of vCJD 8·5 years after receiving a transfusion of red blood cells from a donor who developed vCJD about 17 months after this blood was donated. The donor to this patient also donated the vCJD-implicated blood transfused to the third patient (Editorial Team, 2007).

All four patients received transfusions of non-leucodepleted red blood cells between 1996 and 1999. Since October 1999, leucocytes have been removed from all blood used for transfusion in the UK.

These data therefore demonstrate clearly that non-leucodepleted red cells from asymptomatic individuals incubating vCJD can transmit the infection by blood transfusion to other individuals and that the risk of them doing so is relatively high.

Donor deferral criteria

There has been little substantive change in blood donor criteria since our previous review (Ludlam & Turner, 2005). Whilst other countries continue to defer those who have spent more than a specified cumulative period of time in the UK, within the UK only those considered by the CJD Incidents Panel to be 'at risk of vCJD for public health purposes' on account of exposure to implicated surgical instruments, blood components or plasma products, and those who themselves have received blood components, are deferred (http://www.hpa.org.uk/infections/topics_az/cjd). There is considerable complexity relating to the introduction of similar donor deferral criteria in the context of cell, tissue and organ donation. Broadly, whilst all forms of donation are excluded for patients with CJD or those considered potentially infected, donation of haematopoietic stem cells and solid organs is permitted from those considered 'at risk for public health purposes' and those previously transfused, subject to a risk assessment that weighs the risk of vCJD transmission against the potentially life-saving nature of an otherwise suitable transplant. Donation of other tissues is based on the same donor deferral criteria as blood. Donor deferral criteria remain, however, blunt risk management tools with potential deleterious effects on blood, tissue and organ supply.

Importation of blood components

Since our last report (Ludlam & Turner, 2005), the use of imported methylene-blue treated fresh frozen plasma (FFP) has been extended to all patients under the age of 16 years and to high users. Solvent detergent-treated FFP is recommended for patients undergoing plasma exchange for thrombotic thrombocytopenic purpura on the grounds that there is some evidence to suggest that methylene-blue treated FFP has a deleterious impact on outcome in this patient group (Alvarez-Larran *et al*, 2004). Consideration continues to be given around the possibility of importing FFP and cryoprecipitate for additional groups of patients. Importation of platelets is likely to be impractical given the short shelf-life of these products. However, it may be possible to import red cell concentrates for some groups of patients, for example for children up to 16 years of age. Consideration also has to be given to cost, quality and regulatory requirements and countervailing risks of transmission of other infectious diseases or of component shortages.

Advances in the development of a screening test

As previously noted (Ludlam & Turner, 2005), neither nucleic acid transmission nor immunological responses have been clearly identified in association with transmission of prion diseases, rendering standard molecular and serological screening assays unfeasible. Surrogate markers, such as 14-3-3, S100 and erythroid differentiation-related factor, have thus far proved insufficiently sensitive and specific to be of clinical value. Considerable progress has however been made in the development of assays for the abnormal conformer of prion protein, PrP^{TSE}.

Normal prion protein (PrP^{C}) is a widely expressed 35 kDa 230 amino acid glycosyl-phosphatidylinositol anchored mem-

Immunocapillary electrophoresis was amongst the first methods that claimed to be able to detect PrP^{TSE} in the peripheral blood. The test material is treated with proteinase and subject to a competitive antibody inhibition assay using a labelled peptide (as the competitor) and a monoclonal antibody that recognises both PrP^{TSE} and the peptide (Schmerr *et al*, 1999; Yang *et al*, 2005). The technique has however proved difficult to reproduce in other laboratories and failed to discriminate between infected and uninfected blood samples in a blinded study (Cervenakova *et al*, 2003b).

Epitope unmasking/masking. More success has been achieved with the conformation-dependent immunoassay (CDI), which is predicated on the observation that some PrP epitopes are masked within the PrP^{TSE} aggregate. An increase in signal intensity produced by a labelled monoclonal antibody by a sample denatured using guanidine hydrochloride when compared with the native (un-denatured) sample denotes the presence of PrP^{TSE} (PrP^{C} gives the same signal intensity under both conditions). The sensitivity of the technique is increased through the use of highly sensitive dissociation-enhanced lanthanide fluorescence immunoassay for antibody detection and, in some versions of the assay, the use of PK to reduce background signal (Safar *et al*, 1998, 2002). CDI appears to achieve greater sensitivity than immunoblot (Bellon *et al*, 2003) and, in the format including PK, may approximate the sensitivity of infectivity assays (Bruce *et al*, 2001). In the absence of PK it appears able to detect PK-sensitive forms of PrP^{TSE}, though it remains unclear as to whether these are infectious or not (Bellon *et al*, 2003).

The epitope-protection assay developed by Amorfix uses a chemical modification process which alters epitopes on normal PrP but not those buried within PrP^{TSE} aggregates. The latter are then disaggregated and the conserved epitopes detected using immunodetection methods (http://www.amorfix.com).

PeopleBio have developed an approach where a single antibody is used for both capture and detection steps leading to the blocking of available epitopes by the capture of PrP^{C} but not PrP^{TSE}.

PrP^{TSE}-specific monoclonal antibodies. Several antibodies have now been developed that appear to be specific for conformation-dependent epitopes present in PrP^{TSE} but not PrP^{C} (Korth *et al*, 1997; Paramithiotis *et al*, 2003; Curin Serbec *et al*, 2004; Zou *et al*, 2004). On these, the antibody 15B3, described by Korth *et al* (1997) and manufactured by Prionics, is the best characterised and has proved capable of detecting infectivity in the peripheral blood of scrapie-infected sheep and BSE-infected cattle in the absence of PK digestion (http://www.fda.gov/ohrms/dockets/AC/06/slides/2006-4240S1_9.ppt). Three other antibodies (Paramithiotis *et al*, 2003; Curin Serbec *et al*, 2004; Zou *et al*, 2004) also appear specific to PrP^{TSE} but have not yet been translated to routine assay format.

PrP^{TSE} – specific ligands. A variety of other ligands have been shown to bind selectively to the abnormally conformed molecule. Plasminogen has been proposed as a means of selective binding PrP^{TSE}, but as it can also bind to a variety of other proteins it is therefore unlikely to be sufficiently specific for assay development (Fischer *et al*, 2000).

Polyanionic compounds are known to selectively bind PrP^{TSE} and this property has been employed in the Seprion assay (Lane *et al*, 2003), which uses coated magnetic beads to capture the molecule. The assay is not dependent on PK treatment and is not species-specific provided a suitable detection antibody is used. It is licensed for postmortem diagnosis of BSE and Chronic Wasting Disease and is reported to be able to distinguish between infected and uninfected blood in scrapie-infected sheep and a small number of human samples.

The approach developed by BioMerieux involves PK digestion, precipitation and denaturation followed by reticulation by streptomycin, chemical capture by calyx-6-arene and detection of the macromolecular aggregates by labelled monoclonal antibody (http://www.fda.gov/ohrms/dockets/AC/06/slides/2006-4240S1_9.ppt). Detection of PrP^{TSE} in a small number of plasma samples from scrapie-infected sheep, BSE-infected cattle and CJD-infected humans has been reported.

Adlyfe have developed a third approach utilising a synthetic peptide based on the region of the PrP molecule involved in the PrP^{C}–PrP^{TSE} conformational transition. The peptide sequence is coupled to its mirror image as a palindromic molecule fluorescently labelled at each end. When incorporated into PrP^{TSE} the peptide folds into a hairpin with a beta-sheet conformation and the flurophores stack and change their fluorescence wavelength. Further, the folded ligand induces further molecules to adopt the folded conformation and thus amplifies the signal (Grosset *et al*, 2005). The assay is reported to have discriminated infected from uninfected plasma in natural and experimental scrapie, BSE and CJD.

Chiron have utilised (http://www.fda.gov/ohrms/dockets/AC/06/slides/2006-4240S1_9.ppt) a synthetic PrP polypeptide to capture PrP^{TSE} on magnetic beads with detection by monoclonal antibody in an ELISA format.

Amplification. Two methods have been used to amplify the detection signal. Screening for intensively fluorescent targets utilises double labelled antibodies, more of which bind to PrP^{TSE} aggregates than to PrP^{C} and giving rise to a stronger fluorescence signal (Bieschke *et al*, 2000). Immuno-polymerase chain reaction (PCR) also provides a method of amplifying the signal from an antibody or ligand conjugated to a nucleotide sequence utilising the PCR (Barletta *et al*, 2005).

Two further approaches have been developed that result in the amplification of PrP^{TSE} itself. The first of these, protein misfolding cyclic amplification (PMCA) has given rise to considerable excitement. PrP^{TSE} seeded into an excess of PrP^{C} leads to formation of new PrP^{TSE}. That PrP^{TSE} is then

fragmented through sonication or shaking and leads to a new round of PrP^{TSE} formation (Kocisko *et al*, 1994; Saborio *et al*, 2001). Recurrent cycles therefore of incubation and fragmentation lead to amplification of the original PrP^{TSE} (Castilla *et al*, 2005). Immunoblot and CDI have been used for detection of PrP^{TSE} and infectivity. Studies show that 140 sonication cycles produced an increase in signal intensity of around 6000-fold, whilst a second 'nested' set of 118 cycles with a fresh source of normal PrP led to an approximate 10^7-fold amplification. The technique has proved capable of discriminating infected from uninfected blood from hamsters experimentally infected with scrapie, however there are recent reports of detection of PrP^{TSE} in uninfected animal brain implying the possibility of low levels of abnormally conformed PrP in 'normal' individuals.

A number of cell-based amplification techniques have been described in which the rodent cell lines N2a (Nishida *et al*, 2000), PK-1 (Klohn *et al*, 2003), Rov9 (Birkett *et al*, 2001) and CAD-5 are infectable by natural or experimental strains of scrapie and demonstrate amplification of PrP^{TSE} detected by immunoblot. No cell-based amplification has yet been successfully reported for CJD.

Both these kinds of amplification take several days (PMCA) to weeks (cell-based assays) and would therefore be better positioned as confirmatory rather than screening assays.

Considerations with regard to assay assessment. Whilst the above is not a comprehensive list of all the assays under development, it does provide a flavour of the range and variety of approaches and their relative strengths and weaknesses. Some of these are now approaching the point at which they may be Council of Europe (CE) marked and marketed as potential clinical assays. There are, therefore, a series of further considerations relating to the potential assessment and utility of prion assays prior to clinical implementation.

The required sensitivity is difficult to gauge because the level, spatial distribution and temporal variation of infectivity in the blood of patients with vCJD or healthy individuals with subclinical infection is unknown. The generalizability of experimental data from mouse and hamster experiments to the human condition cannot be assumed (Castilla *et al*, 2006). Moreover, the relationship between infectivity and PrP^{TSE} is complex. Although many authorities believe PrP^{TSE} to be

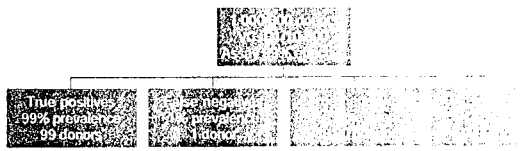

Fig 1. Outcome of screening of a 'normal' population of one million [illegible] variant Creutzfeldt-Jakob disease using an assay with a sensitivity and specificity [illegible] number of false positives greatly exceeds the number of true positives.

[illegible] should not be allowed to continue to donate if they [illegible] it is illegal for example to take the donation [illegible] even if the donor consents to such a [illegible]. Test positive individuals will therefore have to be told of this outcome and (presumably) managed as 'at risk for public health purposes'. Clearly this will cause significant distress and may give rise to psychological and social problems for some people, as well as a disincentive to blood donation and therefore a negative impact on the blood supply. Moreover, it is likely that previous recipients of blood components from donors [illegible] will also have to be traced and contacted (hopefully), giving rise to a much larger group of individuals in the population considered 'at risk of public health purposes' and requiring public precautionary measures to be taken in the event of subsequent medical investigation (see below). A comprehensive health and economic evaluation will therefore have to weigh the positive impact of reducing potential secondary transmission of vCJD against these potential negative consequences.

Blood component processing

Universal leucodepletion was introduced in the UK in 1999 as a measure to reduce the risk of secondary transmission of vCJD. The experimental data from mice infected with the Fukuoka-1 strain of Gerstmann-Sträussler-Scheinker disease (Asante *et al*, 2002) suggests that leucodepletion filters have little impact on plasma-borne infectivity. Studies in the 263K hamster model (Gregori *et al*, 2004) similarly suggest a 4–5 log reduction in whole blood infectivity, consistent with the removal of leucocyte-associated infectivity, but not that present in the plasma. Table I illustrates the likely distribution of residual infectivity in a unit of leucodepleted red cell concentrate prepared by bottom and top processing method (with a residual plasma volume of around 10–15 ml). Assuming 10 ID/ml infectivity in whole blood, just over 130 ID would be left in the unit and that up to a 3 log further reduction is required to impact upon the risk of transmission (i.e. achieve <1 ID/unit). Red cell concentrates prepared by the more common buffy-coat methodology contain greater amounts of residual plasma (around 20 ml) and would consequently require a 4 log reduction. The absence of data on the level of infectivity in human blood means an uncertainty of at least 1 log at any of these point estimates. It can be said in summary, however, that it is unlikely that current blood component processing will suffice to reduce the risk of transmission in more plausible infectivity scenarios.

Three companies are working on the development of prion reduction filters. One has a CE-marked dead-on filter which is used in series with a leucodepletion filter. Published studies using this filter material show >3 log reduction in infectivity on brain homogenate spikes and to the limit of detection (3–4 log) in exogenous infectivity studies (Gregori *et al*, 2006). Two other companies are working on the development of combined leucodepletion/prion reduction filters. All prion reduction filters will have to undergo independent assessment of clinical safety and efficacy within a series of studies managed by the UK and Irish Blood Services and agreed with SEAC and the Advisory Committee on the Safety of Blood, Tissues and Organs (http://www.advisorybodies.doh.gov.uk/acsbto/index.htm). Part of the problem for both manufacturers and Blood Services is the absence of assays capable of detecting either PrP^{TSE} or infectivity in the peripheral blood of patients with vCJD. Assessment of the efficacy of the technology is therefore based on brain homogenate spikes (where baseline infectivity is sufficient to detect a 3–4 log reduction but the physico-chemical form of the spike is unlikely to be similar to that of plasma based infectivity), and endogenous infectivity studies (where the form of infectivity is likely to be more relevant, but the baseline infectivity is sufficiently low that little more than a 1-log reduction is detectable). There remain, therefore, fundamental questions relating to the clinical relevance of different forms of spike material and general applicability of these kinds of studies to the human situation. The potential for deleterious effects on the red cell concentrate itself are also a matter for concern, both in terms of the possibility of alterations to the rheological or antigenic profile of the red cells and the loss in the volume of the additional filter. The latter would have a particular impact if used in conjunction with bottom and top processing, the combined effect of which may reduce the red cell mass in a concentrate below current standards, necessitating additional transfusions for some individuals.

With regard to platelet concentrates, re-suspension in optimal additive solution rather than plasma would reduce the amount of residual plasma by around 65% to 80–90 ml. This would still contain more than enough infectivity to transmit infection to the recipient under even the most optimistic of the current infectivity assumptions and is likely to be ineffectual. Prion reduction filters are not currently applicable to either platelet concentrates or FFP.

Table I. Residual infectivity distribution in a unit of leucodepleted red cell concentrate.

Log reduction in infectivity	Residual leucocytes	Residual plasma	Residual infectivity
Leucodepletion alone	0·2	130	130·2
1 Log	0·2	13	13·2
2 Log	0·2	1·3	1·5
3 Log	0·2	0·13	0·33
4 Log	0·2	0·013	0·213

The data represents the likely distribution of residual infectivity in a unit of leucodepleted red cell concentrate prepared by a bottom and top processing method (with a residual plasma volume of around 10 ml).

Assuming 10 ID/ml infectivity in whole blood with 40% (i.e.4 ID/ml) being removed by leucodepletion and the remainder residing in the plasma (i.e. for a haematocrit of 0·45 a plasma concentration of approximately 13 D/ml), around 130 ID remains in the unit's plasma. Hence up to approximately a 3 log further reduction is required to reduce the risk of transmission to <1 ID/unit.

Plasma product manufacturing

It is reassuring that to date no recipient of a pooled plasma product has developed vCJD. However in 1997, shortly after the first description of vCJD as a new condition, there was concern that the UK plasma supply might have the potential to transmit the infectious agent and that plasma collected from countries where there were few or no cases of vCJD might pose a lower risk (Ludlam, 1997). Although this view gave rise to controversy, the regulatory authorities moved to a position of allowing, and subsequently mandating that pooled plasma products manufactured in the UK should only be made from plasma imported from parts of the world at low risk of vCJD.

In an attempt to help define the risk of PrP^{TSE} transmission by plasma-derived products, detailed studies have been undertaken to assess how prions are partitioned during the plasma fractionation process, mainly by spiking the starting plasma with 'exogenous' prion derived from brain homogenates of experimentally infected animals. The strengths and weaknesses of this approach are similar to those described above in the discussion around the assessment of prion filters. In general there was least clearance of prion in the manufacture of factor VIII, IX and antithrombin concentrates, greater clearance in the preparation of intravenous immunoglobulin, and greatest clearance in the manufacture of albumin (Foster *et al*, 1999).

The way in which different countries responded to the risk that plasma products might transmit the infectious agent varied and depended partly on the perceived relative number of donors who might be infectious as well as details of the plasma fractionation techniques used in each country.

In the UK, using data on partitioning of prion infectivity during manufacture of plasma products, along with the animal data on the likely range of infectivity in individuals with subclinical infection, a risk assessment was undertaken to quantify the risk of recipients of such products being infected. The CJD Incidents Panel have taken the view that an individual with a >1% additional risk of exposure to an infectious dose of vCJD should be notified and managed as 'at risk for public health purposes'.

To date a total of 174 'implicated' batches of plasma products have been identified as having been manufactured from a pool of plasma to which an individual contributed who subsequently developed vCJD (Hewitt *et al*, 2006). For each of these batches a detailed risk assessment was carried out that included the total number of donations included in the pool, the details of the plasma fractionation process used during manufacture and (conservative) estimates of the likely cumulative reduction in infectivity over the manufacturing process. The outcome was expressed as the likely mass of product to which an individual would have had to be exposed to increase

difficulties in the performance of biopsies with gastrointestinal endoscopes because the samples obtained would probably contain lymphoid tissue. The financial implications are significant because the endoscopes cannot be decontaminated and must effectively be discarded. Both upper and lower gastrointestinal endoscopies without biopsy do not result in the instrument being considered as potentially 'contaminated' and it can therefore be reused on other patients after standard cleaning procedure. The concern about possible contamination of instruments has also led to an increased use of capsule endoscopies, which give good images but cannot be used to biopsy or treat gut lesions.

Although no individuals with haemophilia have thus far developed vCJD and a retrospective study of autopsy samples from individuals with haemophilia in 1998 showed no evidence of sub-clinical infection, it has been important to try and gather more data (Lee *et al.* 1998). This has not been easy and depends upon procuring appropriate tissue samples prospectively from individuals undergoing clinically necessary surgery in addition to consent for autopsy. In addition it has been important to try and develop a record of the extent of exposure of individuals to 'implicated' batches of concentrate, as well as all recipients of UK clotting factor concentrates over the 22-year period of exposure. This is being co-ordinated by UK Haemophilia Centre Doctors' Organisation by accumulating the data for subsequent anonymised studies.

Communication with patients and the general public

Keeping recipients of blood and blood products informed about the current state of knowledge and in particular informing individuals about their individual risks has proved challenging because of the complexity and uncertainty inherent in our understanding of the field. It has been important for there to be close collaboration between those able to assess the risk of vCJD infection, physicians responsible for clinical services and patient organisations representing those potentially affected. For those who have received blood components from donors who subsequently developed vCJD, the risk of exposure to vCJD is judged to be high and these individuals have been contacted on an individual basis and offered counselling and specialist follow-up. Similarly, blood donors who have donated blood administered to a patient who later developed vCJD have been contacted and are managed as 'at risk for public health purposes'. In 2004, all patients with haemophilia were sent a letter stating whether or not they had or had not received UK plasma-derived clotting concentrates between 1980 and 2001, irrespective of whether or not they had received UK plasma products, because in an earlier mailing about this topic only those in the 'at risk' group were contacted and this left non-recipients of letters not knowing whether they had not been potentially exposed or whether their letter had got lost in the post. All were offered the opportunity for individual counselling. It is this attention to the detail of how patients are informed that is critical in trying to ensure that individuals feel confident in the arrangements.

For patients potentially exposed to other implicated plasma products, the issue of traceability and notification have proved more problematic. Whilst patients with primary immunodeficiency share a similar close long-term relationship with their physicians, those receiving immunoglobulin for other clinical indications or high doses of albumin (for example during plasma exchange), are often discharged following their acute care. The absence of a general system of traceability for plasma products and of searchable clinical notes has made the follow-up of the latter groups of potentially exposed patients highly problematic.

Concluding remarks

Three years after our last review (Ludlam & Turner, 2005), the management of the risk of transmission of vCJD by blood and plasma products remains highly challenging. Whilst the diminishing number of clinical cases is reassuring, there are continuing uncertainties surrounding the prevalence of sub-clinical disease, the level of infectivity in peripheral blood of such individuals, and the overall risk of transmission and development of clinical disease. Much progress has been made in the development of new technologies, such as prion filters and prion assays, but assessment of these is problematic and cost and countervailing risks need to be considered. Accurate and timely communication with the general public and with those who are considered to be at increased risk of exposure remains essential given the continuing complexity and uncertainty of the field.

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bjh review

Managing the risk of transmission [illegible] disease by blood products

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Summary

Whereas plasma-derived clotting factor concentrates now have a very good safety record for not being infectious for [illegible] enveloped viruses, concern has arisen about the possibility that prion diseases might be transmitted by blood products. There is epidemiological evidence that classical sporadic Creutzfeldt-Jakob disease (CJD) is not transmitted by blood transfusion. There is now good evidence that the abnormal prion associated with variant CJD can be transmitted by transfusion of fresh blood components and infect recipients. To reduce the risk of the pathological prion in the UK infecting recipients of clotting factor concentrates, these are now only manufactured from imported plasma collected from countries where there has not been bovine spongiform encephalopathy (BSE) in cattle and the risk of variant CJD in the population is, therefore, considered negligible. The safety of these concentrates is [illegible] enhanced because prion protein is, to an appreciable extent, excluded by the manufacturing process from the final product. To help reduce the chance of prion transmission by fresh blood products, donations are leucodepleted, there is increasing use of imported fresh frozen plasma (especially for treating children) and potential donors, who have been recipients of blood since 1980 (the beginning of the BSE epidemic in cattle), are deferred.

Keywords: variant Creutzfeld Jakob disease, transfusion, epidemiology, safety, haemophilia.

Emerging pathogens will always challenge the safety of blood transfusion. Whilst the risk of hepatitis B virus (HBV), hepatitis C virus (HCV) or human immunodeficiency virus (HIV) transmission by blood components and plasma products is now small (http://www.eurosurveillance.org), new potentially transfusion-transmissible pathogens continue to emerge.

Many challenges were posed by the emergence of variant Creutzfeldt Jakob disease (CJD) in 1996 (Will *et al*, [illegible]

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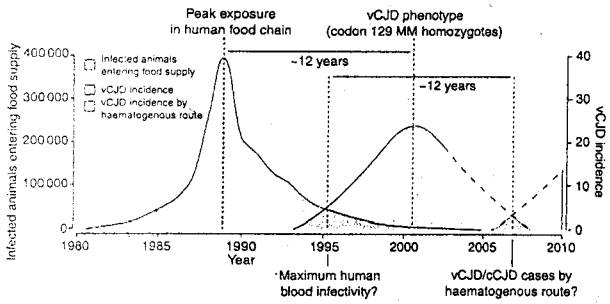

Fig 1. Incidence of bovine spongiform encephalopathy and variant Creutzfeldt Jakob disease in the UK (- - - -, predicted cases). The right hand peak illustrates the potential for secondary spread by haematogenous spread. Reprinted from Collins *et al* (2004) with permission from Elsevier.

addition, a critical polymorphism at codon 129 coding for methionine or valine leads to significant variation in the susceptibility to, and incubation period of, human prion diseases. In the UK, 37% of the general population are homozygous for methionine at this locus, 11% are homozygous for valine and 52% heterozygous. Methionine homozygosity is much more common than expected amongst patients with CJD (*vide infra*). PrP is inserted into the cell membrane predominantly via a glycosylphosphatidyl inositol (GPI) anchor, although transmembrane and soluble forms have also been described. The glycoprotein is predominantly located in calveolar zones in the cell membrane and is estimated to have a half-life of around 6 h, being internalised into endosomes with a proportion recycling to the cell surface (Shyng *et al*, 1993). The function of the protein remains unclear, it has been shown to bind to a laminin receptor precursor protein (Martins *et al*, 1997; Rieger *et al*, 1997) and act as a copper metalloproteinase (Brown *et al*, 1997a). PrP null mice appear to develop normally although some strains show subtle neurological abnormalities (Tobler *et al*, 1996). Prion formation involves changes in the secondary and tertiary conformations of the PrP molecule: up to 40–50% of the molecule can be in the form of beta-pleated sheet, mainly at the expense of the membrane-distal unstructured region. This changes the physicochemical properties of the molecule and engenders relative resistance to proteinase digestion. Prion protein aggregates (PrP^{Sc}) are deposited in cells and tissues leading to the formation of amyloid-like plaques and in the nervous system to neuronal death, astrogliosis and spongiform change.

The mechanism by which PrP^{c} is converted to PrP^{Sc} remains unclear, as does its precise role in the aetiology of the disease. The prion hypothesis (Prusiner, 1998) proposes that the PrP^{Sc} molecule itself converts PrP^{c} to the abnormal conformation, either through a process of heterodimerisation or through nuclear polymerisation (Aguzzi & Weissmann, 1997). PrP^{Sc} is relatively resistant to proteinase-K digestion and different molecular strains of disease can be identified by the balance of di-glycosylated, mono-glycosylated and non-glycosylated species. Several molecular strains of PrP^{Sc} occur in sporadic CJD; however, only a single strain of PrP^{Sc} is found in variant CJD, which is similar to that seen in naturally occurring bovine spongiform encephalopathy (BSE) in cattle, and BSE transmitted naturally and experimentally to other animals (Collinge *et al*, 1996; Hill *et al*, 1997a). Evidence that variant CJD and BSE represent the same strain of prion disease also stems from infectivity studies in a prion disease strain typing panel of inbred experimental mice, where the patterns of incubation period and neuropathological targeting were similar and differed from those seen in sporadic CJD, scrapie and other prion diseases (Bruce *et al*, 1997).

Prion diseases in other species

A range of prion disorders have been described including those involving the SuP35p and Ure2p proteins in yeast, which appear to be non-pathogenic and convey a survival advantage under certain circumstances (Burwinkel *et al*, 2004).

Scrapie was first described as a disease of sheep and goats over 250 years ago and demonstrated to be experimentally transmissible 50 years ago (Aguzzi & Polymenidou, 2004). There is no evidence that scrapie has ever transmitted to man. The only other known self-sustaining animal prion disease is chronic wasting disease in mule deer and elk in several states of the USA. Again there is no current evidence that this disease has transmitted to man.

BSE was first described in UK cattle in 1985 (Wells *et al*, 1987) and is thought to have spread through oral consumption of ruminant-derived meat and bone meal (Wilesmith *et al*, 1988; Brown, 1998). The disease spread widely, peaking in 1992 with over 180 000 clinical cases in the UK, although mathematical estimates suggest that 1–2 million cattle could have been infected but slaughtered and entered the human food chain before they were old enough to demonstrate evidence of clinical disease (Fig 1) (Anderson *et al*, 1996). BSE has crossed into up to 20 other species, including domestic and exotic cats (Wyatt *et al*, 1991; Kirkwood & Cunningham, 1994) and exotic ungulates in British zoos. In July 1988, the spread of BSE led the UK Government to restrict the use of ruminant-derived meat and bone meal as an animal feed and in November 1989 specified that bovine offals were banned for human consumption.

Sporadic Creutzfeldt Jakob diseases

Sporadic CJD was the first described human prion disease, is of uncertain aetiology, has a worldwide distribution and an incidence of around one per million population per year (Will *et al*, 1998). The median age at onset is around 68 years and the disease is characterised by a rapidly progressive dementia leading to death in around 4–6 months. The incidence of the disease varies with the codon 129 genotype of the *PRNP* gene, with 83% of patients homozygous for the expression of methionine at this locus (Deslys *et al*, 1998). Molecular strain typing suggests that six forms of disease are dependent on codon 129 phenotype and strain of prion disease. One of the pathological hallmarks of sporadic CJD is the restriction of accumulation of plaques of prion protein to the central nervous system (CNS). However, with recently developed, more sensitive techniques, prion accumulation has also now been reported to be present in peripheral nerve (Favereaux *et al*, 2004) as well as in muscle, lymphoid tissue and olfactory epithelium (Glatzel *et al*, 2003) at an advanced stage of clinical disease.

Although there are a small number of reports claiming transmission of sporadic CJD by inoculation of blood from patients with clinical disease into experimental rodents (Manuelidis *et al*, 1985; Tateishi, 1985), these results have not been supported by further studies in primates (Brown *et al*, 1994). Similarly, although there are a handful of reports of sporadic CJD arising after blood or plasma product transfusion (Klein & Dumble, 1993; Creange *et al*, 1995, 1996; de Silva, 1996; Patry *et al*, 1998), in none of these has a causal link to a donor with CJD been established. Moreover a series of epidemiological case control (Kondo & Kuroiwa, 1982; Davanipour *et al*, 1985; Harries-Jones *et al*, 1988; Will, 1991; Wientjens *et al*, 1996; Van Duijin *et al*, 1998; Collins *et al*, 1999), look-back (Esmonde *et al*, 1993; Heye *et al*, 1994; Operskalski & Morley, 1995) and surveillance (Evatt, 1998; Evatt *et al*, 1998; Lee *et al*, 1998) studies carried out over almost 25 years have failed to demonstrate evidence of transmission of sporadic CJD by blood components or plasma products. It seems likely therefore that the preclinical incubation period in sporadic CJD is sufficiently short, or peripheral blood infectivity is sufficiently low, as to make transmission of the disease by blood components and/or plasma products at worst a very rare event (de Silva & Mathews, 1993; Brown, 1995; Ricketts *et al*, 1997; Will & Kimberlin, 1998).

Thus, although individuals suspected of having sporadic CJD are permanently deferred from blood donation, no other precautions, such as withdrawal of plasma products if the donor has contributed to the plasma pool, are undertaken. This is because although sporadic [illegible] large number of donors is exposed [illegible] to frequent with drawal and [illegible]

Familial human prion diseases

Familial human prion diseases [illegible] Gerstmann–Sträussler–Scheinker [illegible] insomnia and familial CJD, [illegible] in the prion gene (*PRNP*). All [illegible] familial human prion disease transmit [illegible] individuals with two or more [illegible] disease, or who have been advised [illegible] disease as a result of *PRNP* gene [illegible] blood donation as a precautionary [illegible]

Acquired human prion diseases

The transmission of human prion [illegible] reported in the fore parade of Papua [illegible] 1950s (Gajdusek & Zigas, 1957) [illegible] transmitted during ritual cannibalistic [illegible] rites. The clinical features [illegible] with more prominent cerebellar [illegible] one time Kuru was a leading cause [illegible] people and into the late [illegible] around 1960, [illegible] clinical disease [illegible] period in prion disease [illegible] Iatrogenic transmission of [illegible] direct inoculation of the [illegible] surgical instruments [illegible] mater and corneal [illegible] occurred via neurosurgical procedures [illegible] gonadotrophins [illegible] (Buchanan *et al*, 1981; Brown *et al*, [illegible] presentation varies depending [illegible] centrally transmitted cases [illegible] period of around 2 years [illegible] dementia [illegible] transmitted cases [illegible] have a [illegible] incubation period of around 15–[illegible] ataxia and sensory disturbance [illegible]

Variant CJD

Variant CJD was first described [illegible] 1996) as a result of systematic [illegible] clinical phenotype of CJD in the [illegible] Surveillance Unit in Edinburgh. [illegible] unusual in that it presents with [illegible] such as anxiety or depression [illegible] develop progressive dementia, [illegible] with an average [illegible] (Will, 2003; Will & [illegible]

Table I. Iatrogenic transmission of Creutzfeldt Jakob disease.

	Number	Incubation period (months)
Neurosurgical instruments	5	12–28
Intracerebral electrodes	2	16–20
Dura mater graft	120	18–216
Corneal graft	4	16–320
Human growth hormone	142	550–456
Human gonadotrophin	5	144–192

The incubation period for infections transmitted by peripheral inoculation is shorter than that when infection is directly in the brain (from Ironside and Head, 2003, with permission from Blackwell Publishing).

pathogram changes are observed, but magnetic resonance imaging (MRI) is more informative, with changes in the pulvinar (posterior thalamus) in the majority of cases.

Neuropathologically, the disease is characterised by neural cell loss, astrogliosis and spongiform change with particularly florid amyloid plaques as a pathognomic feature (Fig 2) (Ironside & Head, 2003; Peden & Ironside, 2004). To date all clinical cases of variant CJD have occurred in methionine 129 homozygous individuals; it seems likely that valine homozygous and methionine/valine heterozygous individuals are more resistant to infection or, if infected, to the development of clinical variant CJD. In this context it may be relevant that methionine 129 human prion protein oligomises more rapidly with beta-sheet formation whereas 129 valine tends to form alpha-helix rich monomers (Tahiri-Alaoui *et al*, 2004). Furthermore it is of interest that following inoculation with prions, mice homozygous for human methionine developed 'typical' variant CJD, whilst those that were homozygous for valine appeared more resistant to infection and when this occurred, the clinical and pathological features were more similar to sporadic CJD (Wadsworth *et al*, 2004). It is noteworthy, in this context that the second case of probable variant CJD prion transmission by blood transfusion was recorded in a methionine/valine heterozygous patient who did not develop clinical features of the disease despite surviving 5 years after transfusion (Peden *et al*, 2004). This patient had been identified as part of the variant CJD lookback process and postmortem examination was requested following death from unrelated causes (*vide infra*).

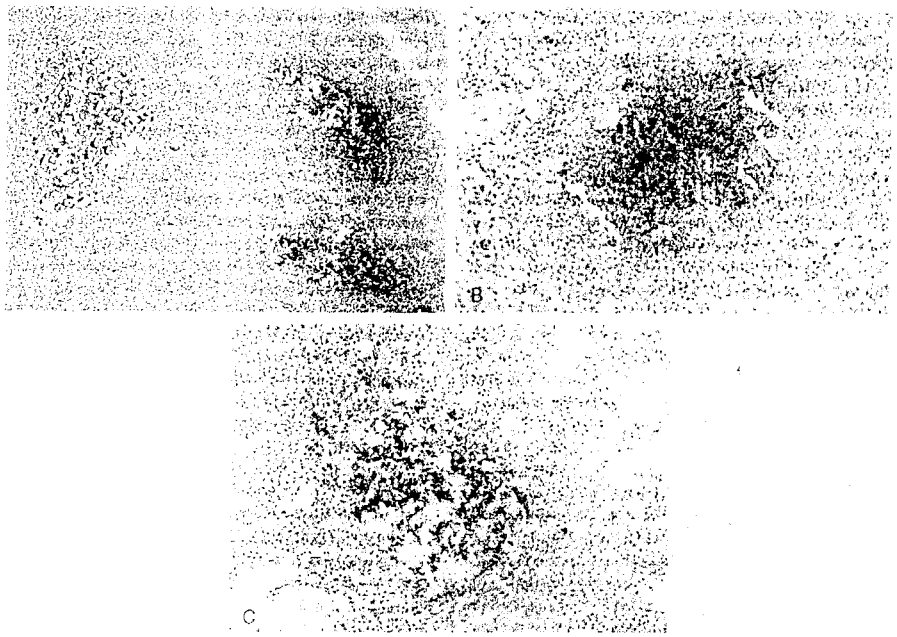

Fig 2. Immunocytochemistry for the prion protein (PrP) in lymphoid tissues in variant Creutzfeldt Jakob disease shows staining of follicular dendritic cells and macrophages in (A) the tonsil, (B) spleen and (C) lymph node. Anti-PrP antibody (KG9) with haematoxylin counterstain [from Ironside and Head (2003) with permission from Blackwell Publishing].

Unlike sporadic and familial forms of CJD, patients with variant CJD show evidence of abnormal prion accumulation in follicular dendritic cells in peripheral lymphoid tissue including tonsils (Hill *et al*, 1997b; Kawashima *et al*, 1997), appendices, spleen (Hilton *et al*, 1998) and lymph nodes (Hill *et al*, 1999). In two patients, appendices removed 8 months and 2 years prior to the onset of clinical disease have also shown evidence of prion accumulation, although a sample removed 10 years prior to onset of clinical disease did not (Glatzel *et al*, 2004).

The median age at death is 29 years (range 14–74 years) and has not altered over the first 10 years of the outbreak, suggesting an age-related susceptibility or exposure (Ghani *et al*, 1998a; Boelle *et al*, 2004). At the time of writing there have been 154 definite and probable cases of variant CJD in the UK, nine in France, two in Ireland and one in each of the USA, Canada, Italy, Saudi Arabia and Japan. In the UK, the incidence of clinical disease appears to have peaked around 2000 and has since fallen significantly (http://www.cjd.ed.ac.uk). However, although the outbreak thus far has been very much less than that which was initially feared (Cousens *et al*, 1997; Ghani *et al*, 1998b), with an upper boundary of around a further 70 new cases now predicted based on the pattern of clinical disease (Will, 2003; Smith *et al*, 2004; Sneath, 2004), a recent retrospective study of tonsil and appendix samples demonstrated three of 12 500 samples positive for abnormal prion accumulation, suggesting that up to 3500 people could be infected with a prevalence of pre- or subclinical disease amongst the 10 to 30-year-old UK population of one of 10 000 (Hilton *et al*, 2004). Ghani *et al* (1998a) have suggested that up to 90% of individuals infected may have prolonged preclinical or true subclinical disease and that this could be related to codon 129 genotypes encoding valine homozygosity or methionine/valine heterozygosity. If transmissible prion infectivity is present in the peripheral blood of such asymptomatic individuals, the concern is that blood-derived products could provide a route to long-term persistence of variant CJD within the population.

Animal studies of peripheral blood infectivity and transmissibility

The route by which the prions disseminate and replicate following peripheral inoculation is of importance in understanding the likely distribution of infectivity and has been recently reviewed (Mabbott & Turner, 2005). Studies in knockout mice with deficiencies in PrP expression, or lacking various cellular compartments of their immune systems, have led to the conclusion that initial accumulation or replication in follicular dendritic cells is essential to peripheral transmission (McBride *et al*, 1992; Bueler *et al*, 1993; Fraser *et al*, 1996; Brown *et al*, 1997b; Klein *et al*, 1997, 1998; Mabbott *et al*, 1998). Indeed, infection and abnormal prion accumulation can be demonstrated in the lymphatic tissues of scrapie-infected rodents and sheep prior

The reverse arm of the surveillance scheme addresses the question as to whether any of the patients who have developed variant CJD could have become infected via a previous blood transfusion. The transfusion history of all patients developing variant CJD is assessed and the donors are traced and also flagged to the UK Office of National Statistics.

To date 17 variant CJD patients are known to have been blood donors (15 in the UK and two in France). Of the 50 recipients of blood components, 17 are still alive. Plasma from 23 donations was fractionated to produce albumin, immunoglobulin and clotting factor concentrates that were used in the UK, France, Belgium, Germany and Italy. In the UK it appears that the incidence of variant CJD peaked in about 2001 and is now declining (Fig 1).

To date there have been two cases of probable transmission of variant CJD prions via non-leucodepleted red cell concentrates. In the first episode, a 24-year-old individual gave a blood donation in 1996 (Llewelyn *et al*, 2004). Three years later he developed variant CJD and died the subsequent year. The recipient of this donation in 1996 was aged 62 years and also received four other units of red cell concentrate to cover a surgical operation. In 2002 he became depressed and developed blurred vision, motor difficulties including a shuffling gait and cognitive impairment. An MRI of his brain was reported as normal. In 2003 he died of dementia. At autopsy, histology of his brain revealed characteristic features of variant CJD, and this was confirmed by proteinase-K resistance and typical features on Western blotting. Analysis of his *PRNP* gene revealed him to be homozygous for methionine at codon 129. A statistical assessment concluded that there was only a 1:15 000–1:30 000 chance of this occurring by coincidence.

A second individual was reported in 2004 as a result of the national surveillance of recipients of transfusions from donors who later developed variant CJD. This patient very likely became infected with variant CJD prions by a unit of red cell concentrate in 1999 from a donor who developed variant CJD 18 months later (Peden *et al*, 2004). Although this patient died 5 years after the transfusion of unrelated causes with no clinical features of variant CJD, analysis of her lymphoid tissue at autopsy revealed that prion accumulation was present in the spleen and one cervical lymph node. There were no histological features or evidence of prion accumulation in her CNS. The other unusual feature as noted above, was that the *PRNP* gene was heterozygous at codon 129 for methionine/valine.

These two cases are therefore of great importance because they have demonstrated that variant CJD prions can be transmitted by blood transfusion from donors who are in a preclinical phase of disease at the time of donation and that methionine/valine heterozygous individuals can also be infected, although whether they are as susceptible to infection and/or the development of clinical disease as methionine homozygous individual remains uncertain (Aguzzi & Glatzel, 2004).

Blood donor selection

Many countries have instituted policies of donor deferral for those who have spent time in the UK, France or more broadly Europe, based on the likely comparative level of risk with their indigenous population, the extent or pattern with which their population visit affected areas and the likely impact on their blood donor base.

In the UK, there are few epidemiological criteria that would allow identification of a 'high-risk' donor population. In response to the blood transfusion related transmissions of variant CJD, in 2004, a policy of deferral of donors who themselves have been recipients of blood components since 1980 was instituted to reduce the risk of tertiary or higher-order transmissions leading to a self-sustaining outbreak. This policy also has the advantage of reducing the risk of other blood borne infectious agents being recycled in the community by transfusion. There was concern that this would lead to a significant reduction in the donor base and that a sometimes precarious blood supply would be further compromised. Whilst about 5–10% donors have been lost from the UK blood donor panels, the impact has been mitigated by proactive recruitment campaigns to enlist more new donors.

Importation of blood components

It is not likely to be feasible to import red cell or platelet concentrates due to the large volumes required, the short shelf life and lability of these components and concerns over the risk of other transmissible agents in some overseas donor populations. To reduce the risk of variant CJD transmission to children, in 2002 the decision was made to only use imported non-UK plasma to treat those born after 31 December 1995. This date was chosen because it was considered that BSE-infected foods had been largely eliminated from the diet by this date, and therefore, children born after this time were unlikely to be infected from food. In addition, with relatively small volumes of plasma, the product can be stored, transported frozen and be virus-inactivated.

Donor screening

No immunological response to prion infection has yet been identified nor has DNA been found associated with disease transmission. Therefore, traditional serological and molecular biological approaches to donor screening are not currently feasible.

Several groups have looked at the possibility of using surrogate markers. The proteins 14·3·3 (Zerr *et al*, 1998) and S100 (Otto *et al*, 1998) are non-specific markers of CNS damage and are therefore likely to be elevated only in the clinical stages of disease. It has been shown that transcription of erythroid differentiation associated factor (EDAF) is depressed in the peripheral blood of animals suffering from prion disease (Miele *et al*, 2001). The cause of this observation is uncertain and it also currently remains unclear whether this could be translated into the setting of human clinical and preclinical disease and whether an appropriate differential exists between patients incubating variant CJD and normal individuals.

Infectivity has not thus far been detected in the peripheral blood of patients with clinical variant CJD by intracerebral inoculation into rodents despite the evidence of clinical transmission, reflecting the limitations of infectivity bioassays due to the species barrier and the small amounts of blood inoculated.

A central difficulty in the development of molecular assays is the differentiation of PrP^{Sc} from PrP^{c} (Minor, 2004). There are currently no monoclonal antibodies or other reagents of sufficient analytical specificity to differentiate between the normal and abnormal isoforms. Most assays therefore depend on differential physicochemical characteristics, such as resistance to proteinase-K digestion or display of additional or novel PrP epitopes following treatment with chaotropic agents, such as guanidine hydrochloride. The level of sensitivity required is challenging. Brown *et al* (1999) has estimated that in the order 1 pg of PrP^{Sc}/ml may be present in the peripheral blood of individuals in the pre- or subclinical phases of disease, in the context of around 100 ng/ml of PrP^{c}, i.e. a ratio of 1 PrP^{Sc} molecule:1 million PrP^{c} molecules. There are also significant challenges in validating such assays. This would normally be undertaken using samples from individuals with the disease in question. However, there are very few patients alive at any one time with variant CJD and large amounts of blood cannot be drawn for ethical reasons. As it is not currently possible to determine who may, or may not, be incubating the disease, the assays will therefore need be validated on brain homogenate-spiked human blood or animal endogenous infectivity samples posing questions around the extrapolation of the data to the human setting. Finally it should be borne in mind that it will not be possible to determine which of the donors with positive assays are actually incubating variant CJD and which of these are likely to go on to develop clinical disease. There is no treatment available at the present time to offer such individuals. There is concern, therefore, over the number of donors who may need to be deferred due to positive assay results and the potential impact of the introduction of such assays on the willingness of donors to donate (Blajchman *et al*, 2004; McCullough *et al*, 2004).

Blood component processing

In October 1997, the UK Spongiform Encephalopathy Advisory Committee advised that universal leucodepletion be considered. The UK Departments of Health commissioned an independent risk assessment by Det Norske Veritas Consulting (DNV) and asked the Blood Services to consider the feasibility (Comer & Spouge, 1999). Implementation was recommended in July 1998 and completed by the autumn of 1999 (Department of Health, 1998a,b). The measure was predicated on st[illegible] to be involved [illegible] leucocytes wer[illegible] peripheral blood [illegible] animal studies [illegible] plasma and is lik[illegible] by only about 4[illegible] St Romaine *et al*, 2004[illegible] considered to [illegible] reduction in [illegible] cytomegalovirus and human T-cell lymph[illegible] of alloimmunisation, immunomodulatory effe[illegible] sion-mediated graft *versus* host disease [illegible]

Other approa[illegible] infection risk in[illegible] a single donor [illegible] donors), exten[illegible] cryoprecipitate [illegible] further reducti[illegible] concentrates.

Two compan[illegible] reduce the pri[illegible] magnitude. [illegible] likelihood of [illegible] sub- or precli[illegible] a significant [illegible] naturally inf[illegible] therefore ha[illegible] enised infect[illegible] infected rode[illegible] these models.

Plasma product manufacture

In 1997, the Committee for Proprietary [illegible] (Committee for Propriety Medicinal Pro[illegible] licensing authority, recommended recall of [illegible] date, factor VIII concentrate that had been man[illegible] plasma pool containing donations from two [illegible] subsequently developed variant CJD. The UK [illegible] Centre Doctors Organisation (UKHCDO) [illegible] recommended that as variant CJD occur[illegible] the UK, it is likely that any risk of transmissi[illegible] by using concentrate prepared from [illegible] collected in other countries [illegible] of variant CJD [illegible] Ludlam, 1997). The UK[illegible] keen to try to evaluate the risk of blood transm[illegible] CJD so that other national safety measures [illegible] 2004, the original DNV risk assessment was [illegible] further estimat[illegible] quantitative risk that [illegible] animal studies [illegible]

Although [illegible] degradation [illegible] severe to [illegible] feature [illegible]

plasma fractionation process (Foster, 1999). Studies with blood from endogenously infected animals (Brown *et al*, 1998; Foster, 2000), and blood spiked with high-titre brain homogenates (Foster *et al*, 2000, 2004; Tateishi *et al*, 2001; Reichl *et al*, 2002; Stenland *et al*, 2002; Vey *et al*, 2002), suggest that a number of steps in existing plasma fractionation processes should contribute individually to reduction in infectivity, including cryoprecipitation and cold ethanol fractionation, depth filtration, adsorption chromatography and nanofiltration. Some of these steps have also been studied in sequence, where it has been shown that, in general, the overall degree of prion removal exceeds that of any one individual step but is less than the sum of the individual steps (Foster, 2004).

Other measures

For the past 5 years the UK Transfusion Services have had an active policy of trying to optimise the use of all blood and blood products. An important component of this policy has been to ensure the appropriate use of red cell concentrates. The aim has been to prevent unnecessary red cell use as exemplified by Stehia *et al* (1994). Such a policy not only reduces the risk of all transfusion-transmitted infections to each individual patient but it allows more patients to be treated with a scarce red cell resource.

Non-blood transfusion related strategies to prevent secondary spread of variant CJD horizontally in population

Between 1996 and 2004 several attempts were made to assess the risk of horizontal spread of variant CJD transmission by mechanisms other than blood products and make rational recommendations on appropriate safety measures (Bird, 2004). There has been concern about transmission in health care settings by invasive medical and surgical procedures. The second 2004 DNV risk assessment was informed by animal studies, which provided some measure of risk related to prion load in the inoculum. The aim was to try and identify the patients and procedures for which specific safety precautions should be instituted. Clearly some level of precaution was appropriate for patients who had clinical variant CJD, but for what other groups of individuals should precautions be taken? It was proposed that precautions should be taken for individuals who could be identified as having more than a 1% risk of exposure to an infectious dose of variant CJD prions (two ID_{50} extrapolated from experimental rodent studies).

The UK CJD Incidents Panel and Health Protection Agency offered advice based on the 2004 DNV risk assessment in relation to recipients of blood components and plasma products. Precautions were to be taken with all identified recipients of fresh blood components from donors who went on to develop variant CJD. For those who received fractionated plasma products, the risk from each was calculated on a product-by-product basis, dependent on the size of the donor pool, detail of the manufacturing process, and the dose of product that would give a 1% risk of exposure to an infectious dose (as defined above) was estimated. The products were divided into three groups based on the assessed risk. Those that were considered to pose a high risk were factors VIII/IX and antithrombin concentrates, where less than one injection of a therapeutic dose for an adult would exceed the risk threshold. Products in the medium risk group were those where the risk threshold would be exceeded if several or more treatments were given and included intravenous immunoglobulin and high doses of albumin. The low risk group consisted of products where very high doses, far in excess of those used in normal medical practice would be required to exceed the risk threshold, e.g. albumin used as an excipient in other products, intramuscular immunoglobulin.

Having defined the threshold dose of 'implicated' product it was necessary to identify which patients were likely to have received such a dose. For those with haemophilia and antithrombin deficiency, it would have been possible in principle to have identified all those patients known to have received implicated concentrates. But this was likely to represent a significant proportion of all UK haemophiliacs as, by September 2004, 16 batches of factor VIII and eight batches of factor IX were implicated and furthermore, it is likely that more batches used in treatment several years ago will become implicated as further former blood donors develop variant CJD in the future. It was therefore decided to use a 'population' approach and consider all haemophiliacs who had received clotting factor concentrate manufactured from UK plasma between 1980 (the beginning of the BSE epidemic) and 2001 (the expiry date of the last batch of product prepared from UK plasma) as being 'at risk of variant CJD for public health purposes'. Such a policy strongly, advocated by UKHCDO, was seen as the simplest and least threatening way to categorise those for whom extra precautions would need to be taken for certain invasive procedures. For other groups, e.g. those with immunodeficiency, patients are being reviewed individually and a decision made as to whether they would fall into the 'at additional risk of exposure to variant CJD for public health purposes' category (Hewitt, 2004).

For those considered to in the 'at additional risk of exposure to variant CJD for public health measures' group, either on the basis of population or individual assessment, the arrangements to prevent horizontal transmission have been laid out by the Advisory Committee on Dangerous Pathogens (http://www.hpa.org.uk/infections/topics_az/cjd/blood_products.htm). In such individuals CNS tissue constitutes a high risk of tissue infectivity and therefore potential contamination of surgical instruments. Surgery on lymphoid tissue or olfactory epithelium and the anterior chamber of the eye, e.g. cataract surgery, involved tissue of medium risk infectivity. Instruments for all these procedures should either be disposable or 'quarantined' after surgery and not reused. It has been suggested that some of these could profitably be used for research studies into decontamination techniques. All other surgeries, including dental and orthopaedic, were not considered to pose a significant risk of contaminating instruments with prions as the tissues were considered at low risk of infectivity and therefore no special precautions were advised.

With the publication of the primate study (Herzog *et al*, 2004), in which, following infection of Macaques with BSE prion both orally and intravenously, PrP^{Sc} was clearly demonstrated in the gut subepithelial neural plexuses as well as Payer's patches, it became clear that endoscopic biopsies of the gut mucosa could potentially contaminate the biopsy forceps and its channel in the instrument with PrP^{Sc}. Whilst the current recommendation is that endoscopes used for non-invasive procedures be cleaned and reused in the normal way, those used for invasive procedures, e.g. colonic biopsies, should be 'quarantined' and not reused. This has had major financial implications for hospitals.

Concluding remarks

Management of the risk of transmission of variant CJD and indeed, other prion diseases by blood and plasma products remains highly problematic (Wilson & Ricketts, 2004a,b). Although the relatively small and falling number of clinical cases in the UK is reassuring, data indicating that up to 98% of infected individuals may sustain long-term preclinical or subclinical disease and that most such individuals are likely to be currently in the 20–40 years age group suggests a significant pool of potentially infectious blood donors. Blood donor selection criteria are a blunt instrument for risk management and current measures, such as universal leucodepletion, seem likely to be only of limited efficacy. Blood donor screening assays and prion reduction filters offer a better chance of control, but much of the validation will need to be based on animal experimentation, the extrapolation of which to the human setting is problematic. Most new risk reduction measures are likely to be highly expensive and engender the possibility of alternative risks, including critical blood shortages. In this context, it is of increasing importance that health services work to ensure prescription of blood products only where they are required (Hart *et al*, 2004; McClelland & Contreras, 2005).

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Vaccines, Blood & Biologics

Questions and Answers on "Guidance for Industry: Revised Preventive Measures to Reduce the Risk of Transmission of Creutzfeldt-Jakob Disease (CJD) and Variant Creutzfeldt-Jakob Disease (vCJD) by Blood and Blood Products"

Why do we recommend new blood donor deferrals for possible exposure to BSE and vCJD?

FDA is taking this step as a prudent measure to protect the blood supply by further reducing the theoretical risk that vCJD may be transmitted by blood transfusion. FDA previously recommended the first donor deferral steps to reduce the risk of exposure to the vCJD agent, which is believed to be the same agent that causes bovine spongiform encephalopathy (BSE, or "mad cow disease"). These steps included deferral of donors who resided in the United Kingdom (U.K.) for a cumulative period of 3 months or more between 1980 and 1996. At this time, we are recommending two additional donor deferrals for possible exposure to BSE and vCJD for the following reasons:

1. Since 1999, the rate of vCJD cases in the U.K. has declined substantially.
2. Significant exposures to potentially contaminated beef products occurred in France and cases of vCJD have appeared in France.
3. Significant exposures to potentially contaminated beef products occurred at U.S. military bases in Europe.
4. In Europe, outside the U.K., the BSE agent has been found in cattle.
5. Particularly in the U.K., transfusion recipients have developed vCJD, presumably due to donors already infected with vCJD.

What are the new donor deferrals?

1. Residence in the U.K. for a cumulative period of 3 months or more between 1980 and 1996.

Rationale: The U.K. has experienced the majority of cases of vCJD (over 150 cases) and has the largest number of cases of vCJD (over 150 cases). Additionally, the U.K. instituted and enforced rules to prevent cattle that were infected with BSE from entering the human food chain (www.defra.gov.uk/foodrin/bse/index.htm; www.food.gov.uk/health/public-health-index.html). Due to these efforts to reduce the risk of BSE and other food protections, the risk of exposure to the BSE agent has been greatly reduced in the U.K. For this reason, the donor deferral extends only through 1996.

2. Military personnel (current and former), and their dependents, who have spent time in military bases in northern Europe, 1980-1990, or Europe (1980-1996), for 6 months or more.

Rationale: British beef was eaten at military bases during these time periods. The maximum amount of U.K. beef eaten was about 35% of the total beef diet.

3. Donors who lived in France for 5 years or more, between 1980 and the present.

Rationale: The French imported at least 5% of their beef supply from the U.K. before 1996. There are also 5 cases of vCJD in France. This deferral will go into place before the European deferral (# 5., below).

4. Donors who received a transfusion in the U.K. between 1980 and the present.

Rationale: Although there are no known cases of transfusion of vCJD, it is too early to rule out this possibility. Since the U.K. has the highest number of vCJD cases, and is likely to also have the highest number of people incubating vCJD, we recommend deferral of people who have received blood products from U.K. donors.

5. Blood donors who lived in Europe for 5 years or more, between 1980 and the present.

Rationale: Most European countries now have reported BSE, although in fewer cattle than in the U.K. However, methods to prevent BSE from getting into human food are not completely in place in all European countries, so we recommend deferral up to the present time.

How effective are the new donor deferrals at reducing risk of vCJD from transfusion?

Combined with the effect of our previous recommendations, our new recommendations, added to the previous U.K. deferral, eliminate an estimated total 90% of overall risk (calculated by "risk-weighted" person-days of exposure to infected beef), and may decrease the number of donors an average of an additional 5% nationwide. The new deferrals reflect an attempt to minimize the theoretical risk of transmission of vCJD, while maintaining critical supplies of blood products.

Why can people who have lived in Europe for 5 years or more, give Source Plasma, but not blood?

Blood donors are deferred, but donors of "Source Plasma," who have lived in Europe (except France and the U.K. as above), may continue to donate. Unlike blood, Source Plasma undergoes manufacturing into highly processed products ("plasma derivatives"), several of which have been in short supply. Donors who have lived in Europe have a low likelihood of incubating vCJD, compared to people who lived in France or the U.K. Furthermore, published studies show that some of the steps used in plasma derivative manufacturing remove agents which are similar to the vCJD agent, thus adding a potential

margin of safety. Thus we consider the risks and benefits of deferral for Plasma donors, as opposed to blood donors, for residence in Europe to be different.

How will the new deferrals affect the blood supply?

Based upon a 1999 survey, we estimate that about 8% of blood donors will be deferred. However, in some locations, such as in large coastal cities where more people travel, up to 10% of donors may be deferred.

What measures are being taken to attenuate the impact of the donor deferrals?

1. We have recommended two separate phases of deferral implementation, to spread out the potential impact on supplies over time. Phase I will start on May 31, 2002, and includes deferral of people who lived in the U.K. for 6 months or more, 1980-1996), in the rest (1980-present) of Europe and military bases (as described above), and who had a transfusion in the U.K. Phase I will provide 82% of the additional risk reduction accomplished by the revised deferral policy and is estimated to eliminate 77% of current potential vCJD risk.

For blood donors who lived in Europe for 5 years or more, deferral will start on October 31, 2002. Phase II will provide the balance of the additional risk reduction accomplished by the revised deferral policy and is estimated to eliminate an additional 13% of current potential risk.

2. We have asked blood banks that choose to have broader deferrals than those we recommend, to implement pilot studies, to see what level of loss of donors can be tolerated without causing local blood shortages.
3. The Department of Health and Human Services has established a system for monitoring the blood supply, nationwide, in an effort to detect supply shortages.
4. We continue to encourage more blood donations, and encourage among blood banks to assist each other in cases of local shortages.

If I am deferred, will I ever be able to donate again?

Because it is still uncertain whether blood can transmit vCJD, and because it is possible that donor screening tests may be developed to identify blood carrying the disease, it is possible that you will be able to donate in the future. Along with our expert Transmissible Spongiform Encephalopathy Advisory Committee (TSEAC), we are continuing to monitor the BSE epidemic, human exposure to BSE, possible testing methods, and scientific advances which will help us understand whether or not human blood components are able to transmit vCJD. New advances in science and epidemiology may enable you to donate again in the future.

What will happen when new **countries, not now on the blood donor deferral list, are discovered to have BSE?**

Since the publication of our draft guidance in August 2001, BSE was diagnosed in Japan, which is not on the blood donor deferral list. The source of this outbreak is believed to be contaminated material from BSE cattle, which was imported and fed to Japanese cows. The news media has reported that other countries may also have received potential BSE-contaminated material which they could have fed to their own cows. We may consider additional deferrals based upon possible exposure to BSE in Asia or elsewhere, but only after additional information about the potential level of BSE exposure and food chain controls in these other countries is acquired and, preferably, would anticipate doing so after the currently recommended deferrals have been implemented and their impact is assessed.

How is FDA monitoring the **risk of vCJD transmission by blood?**

We monitor the risk by keeping up to date with new published, and unpublished scientific work from academia and industry. Much of this material is made publicly available at meetings of the TSEAC. We maintain close contacts, and consult with experts in other agencies that are also involved in BSE and vCJD, such as the U.S. Department of Agriculture and the Centers for Disease Control and Prevention, as well as with international government agencies. FDA also maintains its own pool of scientific experts in these diseases who perform active research to address questions of transmission of spongiform encephalopathies, such as BSE and vCJD by blood.

Where can I obtain more **information?**

1. Previous TSEAC transcripts, containing discussion and information about many of the issues and decisions, above:

- TSEAC Transcripts, December 18, 1998
- TSEAC Transcripts June 1-2, 2000
- TSEAC Transcripts, January 18-19, 2001
- TSEAC Transcripts June 28, 2001

Referenced Guidance

- Guidance for Industry: Revised Preventive Measures to Reduce the Possible Risk of Transmission of Creutzfeldt-Jakob Disease (CJD) and Variant Creutzfeldt-Jakob Disease (vCJD) by Blood and Blood Products (PDF) (PDF - 93KB)

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Health Canada / Santé Canada

(19)

Donor Exclusion to **Address Theoretical Risk of Transmission** of variant Creutzfeldt-**Jakob Disease (vCJD) through the Blood Supply**

UNITED **KINGDOM, FRANCE &** ***WESTERN EUROPE***

Canada

1. PURPOSE

The purpose of this Directive is to advise all licensed Canadian blood establishments of [illegible] further measures to reduce the theoretical risks of transmission of vCJD through the blood supply. This is to be accomplished by excluding from donating blood all persons who:

- have spent a cumulative period of time of 3 months or more in the United Kingdom (UK) consisting of England, Scotland, Wales, Northern Ireland, Isle of Man, the Channel Islands between the years 1980 to 1996; or
- have spent a cumulative period of time of 5 months or more in France between the years 1980 to 1996; or
- have spent a cumulative period of time of 5 years or more in other Western Europe(WE) consisting of Germany, Italy, Netherlands, Switzerland, Belgium, Spain, Republic of Ireland, Portugal, Denmark, Luxembourg, Liechtenstein, Andorra between the years 1980 and ongoing; or
- have received a transfusion of whole blood or labile blood components in the UK, France or WE between the years 1980 and ongoing.

The period of time of three months or more spent in the UK or France is not based on a combination of time in either country. The period spent in the above noted WE countries considers either the time spent individually in each country or any combination of time spent in the various countries so that cumulatively, the residence period requiring deferral is five years or more .

2. BACKGROUND

Variant Creutzfeldt-Jakob disease (vCJD), first described in 1996, is a "new" disease associated with the outbreak of Bovine Spongiform Encephalopathy (BSE) in cattle.

While there have been no cases of vCJD attributable to the use of human blood or plasma derivatives to date, lack of experience with this condition and the substantive [illegible] limited knowledge available on certain biological effects associated with this infectious agent, lack of information on the concentration and infectivity of the vCJD prion in blood to allow for conclusion that it can not occur. In addition, a report that TSE in sheep can be transmitted within that species through blood transfusion, suggests that these diseases have the potential to spread through human blood [illegible] the Transmissible Spongiform Encephalopathies (TSE) can be transmitted [illegible] incubation period of the known TSE infections [illegible] vCJD and BSE [illegible] diagnostic procedures available for early detection. Consequently, Health Canada [illegible] to mitigate the risks of potential human to human transmission of vCJD with [illegible] donor deferral for persons who have spent time in the UK, or France or WE.

In considering this potential risk and measures to deal with it, the principle has been [illegible] that one must seek to apply measures which will reduce the targeted risk without [illegible] the safety of the blood system in other ways. Using this rationale, Health Canada issued Directives on August 17, 1999 and August 20, 2000 requiring the exclusion from blood donation of all persons who had spent time amounting cumulatively, to a period of 6 months or [illegible]

the UK or France between the years 1980 to 1996, inclusive. Based on recent scientific knowledge available since the issuance of the 1999 and 2000 Directives, Health Canada, in consultation with stakeholders including Canadian Blood Services(CBS) and Héma-Québec(HQ), is directing industry to tighten the blood donor deferral for the UK and France to 3 months or more and to add a deferral based on 5 years or more spent in the above-noted countries of WE.

This new Directive is based on recent scientific knowledge available since the issuance of the 1999 and 2000 Directives and the following new information:

- The total number of cases of vCJD is increasing, with a cumulative total that reached 110 in August, 2001, with 106 in the UK, France reporting 3 cases and one case in the Republic of Ireland;
- The number of observed BSE cases is increasing steadily in West European countries once thought to be free of the disease;
- Brain tissue from BSE-infected primates, injected intravenously into other primates, has been shown to transmit disease;
- Recent research has shown experimental sheep-to-sheep transmission of the BSE agent by blood transfusion.

Recent surveys conducted by CBS and HQ indicate that reducing the deferral period to three months or more for either France or the UK and the addition, of a deferral based on 5 years or more time spent in the above-noted countries of WE, will not jeopardize the blood supply. Health Canada's Population and Public Health Branch has carried out a number of modeling studies to estimate the theoretical risk of acquiring vCJD for those persons who have spent time in the UK. Similar modeling studies have been done to estimate vCJD risk for persons spending time in France and the above noted countries of WE. These risks are not identical and consequently, HC would not require a deferral based on a combination of time in the UK with time spent in France; or a combination of times spent between the above-noted WE countries and either the UK or France. However, WE deferral does allow for a combination of times spent among the above-noted WE countries.

A theoretical risk reduction of 72% is achieved under the 1999 and 2000 Directives. With the implementation of the current Directive, there is expected to be an additional 16-18% reduction of the theoretical risk for an estimated overall risk reduction value of 88-90%. A blood donor loss of around 3% or less is estimated under the current Directive.

3. SCOPE

This Directive applies to all Canadian blood establishments that are licensed to fabricate blood and blood components for transfusion or for further manufacture. Products affected by the Directive include all blood components for transfusion with the exception of: autologous donations, peripheral blood stem cells collected for autologous transplants, rare blood types and products derived from USA-sourced plasma.

4. REGULATORY REQUIREMENTS

Blood establishments are requir[illegible] submit [illegible] to amend [illegible] licence. Submissions should be directed to the Blood and Tissues Division of the Biologics and Genetic Therapies Directorate (BGTD) [illegible]

An attachment must be included which indicates the impact at this [illegible] the donor base and plans to mitigate any such effects. Donors are also [illegible] materials to be used in explaining these deferrals to affected donors in order to [illegible] appropriate understanding of the [illegible]

Regarding the withdrawal of prior donations by deferred donors, Health Canada will [illegible] that all available components collected from these deferred donors that have not been [illegible] or pooled for further manufacture be retrieved.

5. COMPLIANCE DATE

The exclusion is to be introduced as soon as operationally feasible, but no later than six months from the date of this Directive.

6. ADDITIONAL INFORMATION

Blood operators will be required to report semi-annually on the impact of the policy on donor bases and the supply of blood.

On an ongoing basis, Health Canada may update the policy in response to [illegible] knowledge. If other cases of vCJD are identified in the public domain, a risk assessment carried out to determine specifically what deferral period will be required.

The Directive, with a list of supporting references [illegible] an HC website.

Questions concerning the "Donor Deferral Policy for Theoretical Risk of Transfusion-variant CJD through the Blood Supply" should be directed to:
Biologics and Genetic Therapies Directorate
Blood and Tissues Division
3rd Floor LCDC Building #6
Postal Locator 0603C
Tunney's Pasture
Ottawa, Ontario
KIA 0L2

7. REFERENCES

Scientific references used in the development of the Health Canada's "Blood agency policy"

1. Monthly statistics on the United Kingdom's CJD [illegible]

http://www.doh.gov.uk/cjd/stats/aug01.htm
and EUROCJD and NEUROCJD: The European and Allied Countries Collaborative Study Group of CJD(EUROCJD) plus the Extended European Collaborative Study Group of CJD(NEUROCJD)
http://www.eurocjd.ed.ac.uk/
2. Monthly statistics on the cases of BSE determined through testing in the European countries. Monthly BSE testing - Cumulative table from January to May 2001 http://europa.eu.int/comm/food/fs/bse/testing/bse_test06_en.pdf - BSE testing - May 2001
and Office International des Epizooties - Number of reported cases of BSE worldwide
http://www.oie.int/eng/info/en_esbmonde.htm
3. Corinne Ida Lasmézas et al. PNAS, March 27, 2001, vol.98(7),4142-4147 "Adaptation of the bovine spongiform encephalopathy agent to primates and comparison with Creutzfeldt-Jakob disease: Implications for human health"
http://www.pnas.org/cgi/doi/10.1073/pnas.041490898
4. Houston F, Foster J.D., Chong A, et al. Transmission of BSE by blood transfusion in sheep. Lancet 2000; 356:999-1000

The modelling studies carried out by Health Canada's Population and Public Health Branch to estimate the theoretical risk of acquiring vCJD under the conditions of the Directive can be found on the Health Canada website with URL:
http://www.hc-sc.gc.ca/sab-ccs/sep2000_BSE_vCJD_slide11_e.html

Health Canada Santé Canada

April 22, 2005

Additional Donor Exclusion Measures to Address the Potential Risk of Transmission of variant Creutzfeldt-Jakob Disease (vCJD) through the Blood Supply

1. PURPOSE

The purpose of this new Directive is to advise all Canadian blood establishments that fabricate blood and blood components for transfusion of the requirement to implement measures to reduce the potential risk of transmission of vCJD through the blood supply to be accomplished by screening and excluding from donating blood, all persons who received a transfusion of whole blood or blood components in France or Western Europe between the years 1980 and ongoing. These new requirements are in addition to those outlined in Health Canada's Directive *Donor Exclusion to Address Theoretical Risk of Transmission of variant Creutzfeldt-Jakob Disease (vCJD) through the Blood Supply UNITED KINGDOM, FRANCE & WESTERN EUROPE* dated August 30, 2001[1].

To summarize the current requirements, risk reduction is to be achieved by excluding from donating blood, all persons who:

- have spent a cumulative period of time of 5 months or more in the United Kingdom consisting of England, Scotland, Wales, Northern Ireland, Isle of Man, the Channel Islands between the years 1980 to 1996; or
- have spent a cumulative period of time of 5 months or more in France between the years 1980 to 1996; or
- have spent a cumulative period of time of 5 years or more in countries of Western Europe of Germany, Italy, Netherlands, Switzerland, Austria, Belgium, Spain, Republic of Ireland, Portugal, Denmark, Luxembourg, and Liechtenstein between the years 1980 and ongoing; or
- have received a transfusion of whole blood or blood components in the UK, France or WE between the years 1980 and ongoing.

2. BACKGROUND

Variant Creutzfeldt-Jakob disease (vCJD), first described in 1996, is a fatal disease linked with the outbreak of Bovine Spongiform Encephalopathy (BSE) in cattle and the consumption of beef and beef products from cattle infected with BSE[2].

Scientific knowledge of the Transmissible Spongiform Encephalopathies (TSEs) has been hampered by the long incubation period of the known TSE infectious agents (e.g. vCJD and BSE) and the lack of diagnostic procedures available for early detection. Consequently, Health Canada (HC) wishes to mitigate the risks of potential human to human transmission of vCJD with policies on blood donor deferral for persons who have spent time or received transfusion of blood or blood components, in the UK, or France or WE.

In considering this potential risk and measures to deal with it, the principle has been adopted that one must seek to apply measures which will reduce the targeted risk without jeopardizing the availability or safety of blood in Canada. Using this rationale, Health Canada issued Directives based on the scientific knowledge available at the time, on August 17, 1999[3], August 20, 2000[4] and August 30, 2001[1]. The first two directives required the exclusion from blood donation of all persons who had spent time amounting cumulatively, to a period of 6 months or more in the UK or France between the years 1980 to 1996, inclusive, based on the BSE epidemic and the occurrences of vCJD in the UK and France. The August 30, 2001 Directive was issued to tighten the blood donor deferral for the UK and France to 3 months or more, to add a deferral based on 5 years or more spent in the above-noted countries of WE, and to add a deferral for donors who received a blood transfusion in the UK, between the years 1980 and ongoing.

The scientific knowledge related to vCJD since the issuance of the 2001 Directive has increased, including the following:

- A study in 2002 demonstrating that scrapie infected asymptomatic sheep could transmit the disease to other sheep by transfusion[5].
- Research indicates that the intravenous route of transmission of BSE is highly efficient[6]
- There have been two recent reports of potential human to human transmission of vCJD by blood transfusion[7,8]. The two blood donors involved did not develop symptoms of vCJD until 40 and 18 months after the donation. One of two recipients of the suspected blood component was a methionine-valine heterozygote (MV) at codon 129 of the prion protein gene (PRNP), contrary to previous data suggesting that susceptibility to vCJD was restricted to the methionine homozygous (MM) PRNP genotype[7].
- There has been an increase in BSE and vCJD cases reported worldwide[9,10,11]. The total number of definite and probable cases of vCJD has reached 168 as of February 7, 2005, with 154 cases in the UK, 9 in France, and one case each in the Republic of Ireland, Canada, Italy and United States[12,13].

3. REGULATORY REQUIREMENTS

Based on the current scientific knowledg[illegible] establishments that are licenced to fabricate [illegible] further reduce the risk of vCJD transmissi[illegible] exclusion of donors who received a blood transfusion [illegible] ongoing, to include France and WE. These [illegible] Licence Amendment Submission to the Biologics and [illegible] review.

An attachment must be included which indicates [illegible] the donor base and plans to mitigate any such effects. [illegible] develop materials to be used in explaining these deferr[illegible] foster an appropriate understanding of these precaut[illegible]

Regarding the withdrawal of prior donations by defer[illegible] that all available components collected from these do[illegible] or pooled for further manufacture, be retrieved.

4. SCOPE

This Directive applies to all Canadian blood establ[illegible] and blood components for transfusion. Products [illegible] components for transfusion with the exception of [illegible] cells collected for transplants, and rare blood types.

It is recommended that Canadian and non-Canadian [illegible] follow the donor exclusion requirements outlined in [illegible]

5. CONSULTATIONS

The scientific finding have been discussed and advised by the Canadian Advisory Committee on Blood Regulation as well as the Canadian Public Advisory Committee. Also, Canadian blood [illegible] been consulted in the development of this Directive.

The blood donor loss as a result of this new [illegible]

6. COMPLIANCE DATE

The exclusion is to be introduced as soon as [illegible]

months from the date of this Directive.

7. ADDITIONAL INFORMATION

Blood operators will be required to report semi-annually on the impact of this policy on their donor bases and the supply of blood.

On an ongoing basis, Health Canada may update its guidance in response to new scientific knowledge.

Questions concerning the " Donor Exclusion to Address Theoretical Risk of Transmission of variant CJD through the Blood Supply" should be directed to:
Biologics and Genetic Therapies Directorate
Centre for Biologics Evaluation
Director's Office
3rd Floor LCDC Building #6
Postal Locator 0603D
Tunney's Pasture
Ottawa, Ontario
K1A 0L2

8. REFERENCES

1. Donor exclusion to address theoretical risk of transmission of variant Creutzfeldt-Jakob disease (vCJD) through the blood supply. Health Canada Directive, 2001
http://www.hc-sc.gc.ca/hpfb-dgpsa/bgtd-dpbtg/blooddeferral_uk_france_we_e.html

2. Corinne Ida Lasmézas et al. PNAS, March 27, 2001, vol.98(7),4142-4147 "Adaptation of the bovine spongiform encephalopathy agent to primates and comparison with Creutzfeldt-Jakob disease: Implications for human health"
http://www.pnas.org/cgi/doi/10.1073/pnas.041490898

3. Directive 99-01: Donor Exclusion to Address Theoretical Risk of Transmission of Variant CJD through the Blood Supply.
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4. Directive D2000-01: Donor Exclusion to Address Theorerical Risk of Transmission of Variant CJD Through the Blood Supply
http://www.hc-sc.gc.ca/hpfb-dgpsa/bgtd-dpbtg/d2000-01_0830_e.html

5. Hunter, N., et al. Transmission of prion diseases by blood transfusion. J. Gen. Virol., 83: 2897- 2905, 2002.

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8. Peden, Alexander, et al. "Preclinical vCJD after blood transfusion in a PRNP heterozygous patient." The Lancet (2004) 364: 527-528.

9. Monthly statistics on the cases of BSE determined through [illegible] in the European countries.
http://europa.eu.int/comm/food/food/biosafety/bse/bsecjd_en.htm

10. Office International des Epizooties - Number of reported cases of BSE worldwide
http://www.oie.int/eng/info/en_esbmonde.htm

11. EUROCJD and NEUROCJD: The European and Allied Collaborative Group of CJD(EUROCJD) plus the Extended European Collaborative Study of CJD(NEUROCJD)
http://www.eurocjd.ed.ac.uk/

12. Statistics on the United Kingdom's CJD cases
http://www.cjd.ed.ac.uk/figures.htm

13. Number of cases of vCJD in France
http://www.invs.sante.fr/publications/mal_transm/vcjd.htm