References

1. Will RG, Ironside JW, Zeidler M, et al. A new variant of Creutzfeldt-Jakob disease in the UK. Lancet. 1996;347:921-925.
2. Hilton DA, Ghani AC, Conyers L, et al. Prevalence of lymphoreticular prion protein accumulation in UK tissue samples. J Pathol. 2004;203: 733-739.
3. Hill AF, Butterworth RJ, Joiner S, et al. Investigation of variant Creutzfeldt-Jakob disease and other human prion diseases with tonsil biopsy samples. Lancet. 1999;353:183-189.
4. Brown P, Cervenakova L, McShane LM, Barber P, Rubenstein R, Drohan WN. Further studies of blood infectivity in an experimental model of transmissible spongiform encephalopathy, with an explanation of why blood components do not transmit Creutzfeldt-Jakob disease in humans. Transfusion. 1999;39:1169-1178.
5. Hadlow WJ, Kennedy RC, Race RE. Natural infection of Suffolk sheep with scrapie virus. J Infect Dis. 1982;146:657-664.
6. van Keulen LJ, Schreuder BE, Vromans ME, Langeveld JP, Smits MA. Pathogenesis of natural scrapie in sheep. Arch Virol Suppl. 2000:57-71.
7. Jeffrey M, Ryder S, Martin S, et al. Oral inoculation of sheep with the agent of bovine spongiform encephalopathy (BSE): 1. Onset and distribution of disease-specific PrP accumulation in brain and viscera. J Comp Pathol. 2001;124:280-289.
8. Houston F, Foster JD, Chong A, Hunter N, Bostock CJ. Transmission of BSE by blood transfusion in sheep. Lancet. 2000;356:999-1000.
9. Hunter N, Foster J, Chong A, et al. Transmission of prion diseases by blood transfusion. J Gen Virol. 2002;83:2897-2905.
10. Goldmann W, Baylis M, Chihota C, Stevenson E, Hunter N. Frequencies of PrP gene haplotypes in British sheep flocks and the implications for breeding programmes. J Appl Microbiol. 2005;98: 1294-1302.
11. Jeffrey M, Martin S, González L, et al. Immunohistochemical features of PrP (d) accumulation in natural and experimental goat transmissible spongiform encephalopathies. J Comp Pathol. 2006;134:171-181.
12. González L, Martin S, Houston FE, et al. Phenotype of disease-associated PrP accumulation in the brain of bovine spongiform encephalopathy experimentally infected sheep. J Gen Virol. 2005; 86:827-838.
13. González L, Martin S, Begara-McGorum I, et al. Effects of agent strain and host genotype on PrP accumulation in the brain of sheep naturally and experimentally affected with scrapie. J Comp Pathol. 2002;126:17-29.
14. Goldmann W, Houston F, Stewart P, Perucchini M, Foster J, Hunter N. Ovine prion protein variant A(136)R(154)L(168)Q(171) increases resistance to experimental challenge with bovine spongiform encephalopathy agent. J Gen Virol. 2006;87: 3741-3745.
15. Kirby L, Goldmann W, Houston F, Gill AC, Manson JC. A novel, resistance-linked ovine PrP variant and its equivalent mouse variant modulate the in vitro cell-free conversion of rPrP to PrP(res). J Gen Virol. 2006;87:3747-3751.
16. Sisó S, González L, Houston F, Hunter N, Martin S, Jeffrey M. The neuropathologic phenotype of experimental ovine BSE is maintained after blood transfusion. Blood. 2006;108:745-748.
17. Hewitt PE, Llewelyn CA, Mackenzie J, Will RG. Creutzfeldt-Jakob disease and blood transfusion: results of the UK Transfusion Medicine Epidemiological Review study. Vox Sang. 2006;91:221-230.
18. Llewelyn CA, Hewitt PE, Knight RS, et al. Possible transmission of variant Creutzfeldt-Jakob disease by blood transfusion. Lancet. 2004;363: 417-421.
19. Wroe SJ, Pal S, Siddique D, et al. Clinical presentation and pre-mortem diagnosis of variant Creutzfeldt-Jakob disease associated with blood transfusion: a case report. Lancet. 2006;368: 2061-2067.
20. Health Protection Agency. Fourth case of variant CJD infection associated with blood transfusion. Health Protection Agency. 2007. http://www.hpa.org.uk/hpa/news/articles/press_releases/2007/070118_vCJD.htm. Accessed January 18, 2007.
21. Peden AH, Head MW, Ritchie DL, Bell JE, Ironside JW. Preclinical vCJD after blood transfusion in a PRNP codon 129 heterozygous patient. Lancet. 2004;364:527-529.
22. Houston EF, Halliday SI, Jeffrey M, Goldmann W, Hunter N. New Zealand sheep with scrapie-susceptible PrP genotypes succumb to experimental challenge with a sheep-passaged scrapie isolate (SSBP/1). J Gen Virol. 2002;83:1247-1250.
23. Schreuder BE, van Keulen LJ, Vromans ME, Langeveld JP, Smits MA. Tonsillar biopsy and PrPSc detection in the preclinical diagnosis of scrapie. Vet Rec. 1998;142:564-568.
24. Andréoletti O, Berthon P, Marc D, et al. Early accumulation of PrP(Sc) in gut-associated lymphoid and nervous tissues of susceptible sheep from a Romanov flock with natural scrapie. J Gen Virol. 2000;81:3115-3126.
25. Valleron A-J, Boelle P-Y, Will R, Cesbron J-Y. Estimation of epidemic size and incubation time based on age characteristics of vCJD in the United Kingdom. Science. 2001;294:1726-1728.
26. Goldmann W, Hunter N, Smith G, Foster J, Hope J. PrP genotype and agent effects in scrapie: change in allelic interaction with different isolates of agent in sheep, a natural host of scrapie. J Gen Virol. 1994;75:989-995.
27. O'Rourke KI, Holyoak GR, Clark WW, et al. PrP genotypes and experimental scrapie in orally inoculated Suffolk sheep in the United States. J Gen Virol. 1997;78:975-978.
28. Jeffrey M, Martin S, Thomson JR, Dingwall WS, Begara-McGorum I, González L. Onset and distribution of tissue prp accumulation in scrapie-affected suffolk sheep as demonstrated by sequential necropsies and tonsillar biopsies. J Comp Pathol. 2001;125:48-57.
29. Clarke P, Will RG, Ghani AC. Is there the potential for an epidemic of variant Creutzfeldt-Jakob disease via blood transfusion in the UK? J R Soc Interface. 2007;4:675-684.
30. Bishop MT, Hart P, Aitchison L, et al. Predicting susceptibility and incubation time of human-to-human transmission of vCJD. Lancet Neurol. 2006;5:393-398.
31. Ironside JW, Bishop MT, Connolly K, et al. Variant Creutzfeldt-Jakob disease: prion protein genotype analysis of positive appendix tissue samples from a retrospective prevalence study. BMJ. 2006;332:1186-1188.
32. Mead S, Joiner S, Desbruslais M, et al. Creutzfeldt-Jakob disease, prion protein gene codon 129VV, and a novel PrPSc type in a young British woman. Arch Neurol. 2007;64:1780-1784.
33. Gregori L, McCombie N, Palmer D, et al. Effectiveness of leucoreduction for removal of infectivity of transmissible spongiform encephalopathies from blood. Lancet. 2004;364: 529-531.
34. Gregori L, Gurgel PV, Lathrop JT, et al. Reduction in infectivity of endogenous transmissible spongiform encephalopathies present in blood by adsorption to selective affinity resins. Lancet. 2006; 368:2226-2230.
35. Castilla J, Saá P, Soto C. Detection of prions in blood. Nat Med. 2005;11:982-985.
36. Saá P, Castilla J, Soto C. Presymptomatic detection of prions in blood. Science. 2006;313:92-94.

TRANSFUSION

Transfusion

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REVIEWS

From mad cows to sensible blood transfusion: the risk of prion transmission by 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 disease (vCJD), is now established. Subjects infected through food may transmit the disease through blood donations. The two nations most affected to date by this threat are the United Kingdom (UK) and France. The first transfusion cases have been observed in the UK over the past 5 years. In France, a few individuals who developed vCJD had a history of blood donation, leading to a risk of transmission to recipients, some of whom could be incubating the disease. In the absence of a large-scale screening test, it is impossible to establish the prevalence of infection in the blood donor population and transfused patients. This lack of a test also prevents specific screening of blood donations. Thus, prevention of transfusion transmission essentially relies at present on deferral of "at-risk" individuals. Because prions are present in both white blood cells and plasma, leukoreduction is probably insufficient to totally eliminate the transfusion risk. In the absence of a screening test for blood donations, recently developed prion-specific filters could be a solution. Furthermore, while the dietary spread of vCJD seems efficiently controlled, uncertainty remains as to the extent of 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" dates back to 1985. It was observed in the United Kingdom (UK),[1] where the epidemic spread widely: by 2001, close to 180,000 animals had been affected by bovine spongiform encephalopathy (BSE). Most likely, the epidemic appeared as a result of feeding of bovine livestock with animal food prepared from residues from slaughtering and quartering, including carcasses of sheep which died from scrapie and cattle affected by a sporadic form of BSE. The use of such animal feed was forbidden in 1988 in the UK and in 1994 in France. On March 20, 1996, the UK Department of Health announced that the BSE agent was transmissible to man. A new human pathology appeared that year: a previously unknown form of Creutzfeldt-Jakob Disease (CJD), transmitted by a variant of the prion, the agent of sporadic CJD.

Unlike other transfusion-transmissible agents, the prion (**P**roteinaceous **I**nfectivity **O**nly) contains no nucleic acids

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 **sc**rapie) 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 recorded in the UK, and this number accounts for the majority of cases worldwide. Mean age of affected individuals was 28 years (range, 12-74 years), with a slight male predominance. The mean duration of the symptomatic period was 14 months (range, 6-40 months). All tested patients were homozygous MM. The most probable origin for the majority of cases was dietary: no risk factor of other kinds of CJD was observed.[16,17] A small number of cases had a history of blood 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 contaminated beef. Importation of contaminated beef into France increased regularly from 1985 to 1995, while the ingestion of these meats decreased in the UK over the same period.[18] However, the level of exposure in France is estimated[19-21] to have been 10 to 20 times lower than that of the UK, with moreover a difference between the two countries in terms of dates of occurrence: the comparison between the number of French cases and that of UK cases (taking into account the year of the beginning of the symptomatic phase) indicates that a maximal incidence of French cases occurred 5 years after the peak of the epidemic in the UK, where the number of recorded cases has regularly decreased since 1999.[17] This temporal gap in the epidemic in the UK and in France is attributable to the period of maximal exposure of the general population in the two countries.

Between 1996 and 2007, 23 vCJD cases have been registered in France, with a mean age of 36 years (range, 19-58 years) and an equal sex ratio (12 males, 11 females). The clinical and genetic characteristics were comparable to those of the British vCJD patients. The mean duration of the symptomatic period was 15 months (range, 8-24 months). All the analyzed cases were homozygous MM, without any risk factor of other kinds of CJD.[17] Short stays in the UK (less than 10-day periods) were mentioned in 3 patients; a fourth one had stayed several times in the UK, for long periods, between 1987 and 1996.

In other countries, vCJD cases remain exceptional. A small number of these cases are attributable to residence in the UK, but there remain a number which appear to have been acquired outside the UK and France: two cases each in Ireland, the Netherlands, Saudi Arabia, Spain, and Portugal and one in each of Italy and Japan.

Several studies have been conducted to estimate the extent of the vCJD epidemic in the UK and in France. A British retrospective study revealed the presence of the abnormal prion in surgically removed appendix in 3 of 12,674 individuals without clinical vCJD:[22] this rate appears higher than suggested by the number of vCJD cases recorded in the general population. From these results, a prevalence of 237 cases per million of British citizens was proposed (95% confidence interval, 49-792). In the most pessimistic hypothesis (i.e., taking into account the highest range of this interval), 41,250 of 60 million individuals would be infected by the abnormal prion. However, the latest count of vCJD cases in the UK is more in agreement with the lower prediction of epidemiologists.

In France, where the level of exposure was lower than in the UK, estimates of 6 to 300 vCJD cases during the next 60 years have been suggested in one study,[19] and 205 cases in another.[23] In 2003, a model of incidence prediction suggested a total number of 33 cases (0-100), with 14 cases (2-30) over the 2004-2005 period, and 11 (1-20) over the 2006-2010 period.[18] These data are compatible with the most recent epidemiologic reports. A recent study predicted 39 (6-99) subsequent cases.[21] The worst case scenario of 300 cases over the next 60 years is, however, maintained in the epidemiologic estimations, in particular to estimate the prevalence of prion infection in the blood donor population.

Measures taken against the vCJD epidemic, with screening for the BSE agent in cattle and elimination of positive animals from the food chain, and the latest epidemiologic observations suggest that a vCJD pandemic of dietary origin is unlikely in the coming years. The unknowns now reside in other sources of contamination, through blood or cellular vectors: blood transfusion, grafts of tissues or organs, or use of medical or surgical instruments contaminated with the abnormal prion. Transfusion transmission is especially feared due to its potential extent. Nowadays, since food contamination, which was the main source of infection, seems fully controlled, has transmission by blood components taken its place? In the UK, as in France, while prions have probably been eliminated from beef, they are likely to be present in the blood of asymptomatic human carriers and transmissible to the recipients of their blood donations. The fear of human-to-human transmission has thus replaced the fear of interspecies contamination.

EXPERIMENTAL BASIS OF vCJD TRANSMISSION BY TRANSFUSION

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

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. Seven recipients are not known to be dead and have survived 7 to 9 years after transfusion. Three cases who developed fCJD (of 5 reported as blood donors) were associated with 11 identified recipients, of whom 2 died within 1 year of blood transfusion and 3 died 3, 10, and 17 years after transfusion. Three recipients not known to be dead have survived 13 to 21 years after transfusion. Among the 97 recipients thus identified, 4 have developed evidence of infection and 3 of these have died of the disease: these all belong to the first group, those exposed to risk of vCJD. Because there is no evidence that either sCJD or fCJD has been transmitted by blood transfusion, none of those recipients have been 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 nonleukoreduced red blood cells (RBCs). There have been no transfusion-associated cases of sCJD or of fCJD: no cases have ever been described in these two latter groups, even in retrospective lookbacks or in case control studies,[43-47] and no such infections have been detected in the blood in experimental animal studies, even in transgenic mice rendered susceptible to the disease, although it is not possible to formally exclude transfusion cases of sCJD, which might have passed unnoticed if they possessed exceptional features and/or a particularly long incubation period.

The first of the four patients infected with vCJD through blood transfusion was a male in the 60- to 70-year age group, who developed the illness in 2002 and died the next year. During surgery in 1996, he received 5 units of nonleukoreduced RBCs, one of which was donated by a young donor who developed vCJD in 1999 and died the following year. Both donor and recipient were MM homozygous. Infection of dietary origin could not be completely excluded in this case (as in the others), but the transfusion was the most plausible explanation, given the age of the recipient which was greater than the median for cases believed exposed through the dietary route, and by the association of this rare disease in both donor and recipient: statistical analysis demonstrated that the possibility these two observations of vCJD would have happened independently, if transfusion was not the source of infection, was in the order of 1:15,000 (and rose to 1:30,000 taking into account the age of the recipient). This first transfusion-associated case in world literature was reported in December 2003.[48]

The second case was an elderly recipient, who died of cardiovascular causes without developing any clinical signs of vCJD. Asymptomatic infection with PrP^{Sc} was established by postmortem examination, which demonstrated the presence of abnormal prion protein in lymphoid tissue (the spleen and one cervical lymph node, but not in the tonsils or appendix), but not in the brain. This patient had been identified as "at risk" since, 5 years previously, in 1999, a nonleukoreduced RBC component had been provided from a donor who died of vCJD 18 months after the donation. The PrP^{Sc} isolated from the spleen had an isoform identical with that observed in cases of vCJD. The donor was an MM homozygote, but the recipient was a heterozygote (MV), which may explain the asymptomatic nature of this case, assigned as "preclinical" or "subclinical" vCJD. Alternatively, the recipient may have developed vCJD at a later date, if survival had been longer. This second case of possible transfusion transmission of vCJD infection was reported in July 2004.[49]

The third case, reported in 2006,[42,50,51] was also one of the cohort of blood recipients who had received nonleukoreduced RBCs and had been notified of their risk. This recipient was in the 20- to 30-year age group and had received transfusion support during a surgical intervention complicated by serious bleeding. The recipient developed vCJD in 2005, 7 years after the transfusion episode, and died 2½ years later. One of the donors presented with vCJD in 1999 (18 months after the donation) and died the following year (21 months after the blood donation). Both donor and recipient were MM homozygotes.

The fourth and last case to date was a recipient who developed vCJD 8½ years after transfusion with RBCs from a donor who presented with vCJD 17 months after donation. This donor was the same as that to case number three. The recipient, genotype MM, died 1 year after presentation.[52]

These cases reported in professional journals (and subsequently in general reports) have led to the risk of transfusion-associated vCJD moving progressively from "theoretical risk" to "possible," then to "probable" and finally "demonstrated." There are a number of unknowns in the variables of risk of blood contamination by vCJD, but the combination of the low prevalence of vCJD in the general population (the estimation of the risk from an individual unit varies between 1:15,000 and 1:30,000 in the UK)[48] and of the high prevalence of vCJD cases in the small group of recipients who have been rendered at risk (and it should be noted that a sizeable proportion of these at-risk recipients have died without surviving long enough to develop an eventual vCJD and were never tested for the presence of infection) makes highly probable a transfusion origin rather than diet. The occurrence of these cases reinforces the theory that the blood of a donor in the asymptomatic phase of the disease could be infective for recipients. This evidence of the transfusion transmissibility of vCJD thus largely justifies the preventative measures previously applied in the UK and in France.

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