Overall Blood Supply Strategy with Regard to Variant Creutzfeldt-Jakob Disease (vCJD)

Report of the Working Group Commissioned by the German Federal Ministry of Health\textsuperscript{a}
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(Update of the Report from August 17, 2001)

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\textsuperscript{b}Last update before going to press.
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Editorial – The Impact of vCJD on a Safe Supply of Blood and Blood Products

One of the most worrying health problems emerging in the past decades is the epidemic occurrence of new forms of certain neurodegenerative diseases. The prototype of this diseases, a characteristic disorder of sheep and goats called scrapie, had been known for centuries. A similar kind of disease in humans, the Creutzfeldt-Jakob disease (CJD), was first described in 1920. It was found out later that this disease can be transmitted by parenteral application of medicines derived from human substances related to the central nervous system, such as dura mater grafts or hormones from human pituitary glands. Due to these albeit rare transmissions, the typical histologic appearance of involved brain tissue, and the clinical picture, the term transmissible spongiform encephalopathies (TSE) was coined.

About 20 years ago, a new initially mysterious disease of cattle was observed in the UK, which was first called ‘mad cow disease’. It was soon found out that it was a new form of TSE, designated bovine spongiform encephalopathy (BSE). It was obviously spread rapidly by feeding material rendered from ruminant carcasses to cattle, resulting in a huge epidemic and tremendous economic losses in the British beef industry. The long-known sheep disease scrapie had never been observed to be transmitted to humans. Thus it was a frightening experience to learn since 1996 that a variant of CJD (vCJD) occurred predominantly in young people, which had obviously to be regarded as a manifestation of BSE in humans.

Many efforts were made within the scientific society to explore and clarify facts concerning origin, course, characteristics and spreading of vCJD. The theory was developed and substantiated that the pathogen behind TSE is a misfolded form of a cellular protein which was named prion. An important task was to develop and optimize methods for detection of prion protein in biologic samples. This is particularly demanding since miniscule amounts of the pathogenic misfolded protein have to be detected in the presence of abundant normal protein. It is also difficult to find appropriate surrogate markers, and there is no detectable immune reaction to TSE. Suitable screening tests, e.g. for blood or organ donors, are not available.

The new disease vCJD brought about a great challenge also for regulatory bodies. This is true not only for the control of the food chain of animals and humans which had to be rigorously cleared from risk materials. Materials derived from cattle are contained in the majority of medicinal products, e.g. as excipients, and precautionary measures were imposed to ensure the safety. After vCJD was first detected, it was immediately clear that a possible secondary infection, i.e. a transmission via human materials used as medicines, had to be considered or even assumed as worst case scenario. Therefore, the task to develop a strategy for a save blood supply in view of vCJD was on the agenda as a priority issue.

Since the situation with respect to BSE and vCJD epidemiology is very different not only between the continents, but even with Europe, it appears wise that each country should base regulatory decisions on its own risk assessment. Any precautionary measures to ensure blood safety should take into account specific national conditions like the particular BSE epidemiology in cattle, the respective epidemiology in men and the kind and status of national blood product supply.

With the task to develop such a strategy, an expert working group was appointed by the German Federal Minister of Health. The group issued a first report in 2001. The present, substantially revised report provides a summary on prion protein diseases with focus on vCJD and its transmission by blood or blood products. It includes recent developments, e.g. the three cases of vCJD transmission by blood transfusions in the UK, and also modeling studies. Finally, it contains conclusions and recommendations for decision makers with responsibility for blood safety in Germany including the feasibility of certain measures discussed in the last years.

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Summary – Overall Blood Supply Strategy with Regard to vCJD

A working group was formed by request of the Federal German Ministry of Health in a letter dated January 26, 2001. Staffs from the Paul-Ehrlich-Institut, the Robert Koch-Institut and the Federal Ministry of Health as well as external experts were members of this working group. On August 17, 2001, the text prepared by this working group was submitted to the Ministry of Health and published after a discussion in the National Advisory Committee ‘Blood’ (Arbeitskreis Blut). In 2004, the working group became active again, with slightly different members in order to evaluate new developments and prepare an update of the above mentioned report made available by this publication.

As per August 7, 2006 there were known to be 162 cases of vCJD in the UK, 20 in France (as per July 28, 2006), 4 in Ireland, 2 in The Netherlands, 2 in the USA, and 1 case each in Canada, Italy, Japan, Portugal, Saudi Arabia, and Spain. Six of the 34 patients with residence outside from the UK, 2 cases each from Ireland and the USA and 1 case each from Canada and France, had spent a long period of time in the UK. A connection with stays in the UK is questionable in 1 case in Japan. It must also not be ruled out that vCJD will be diagnosed in other countries.

New model calculations [1] have resulted in lower estimated values for the overall number of clinical vCJD cases in the UK compared with previously published data, with, however, considerable confidence intervals. On the basis of new estimates, the number of up to 600 cases of vCJD for Germany indicated in the ‘worst case scenario report’ of August 17, 2001 can be considered as too pessimistic.

Transmissibility by blood was already assumed in the previous report of August 17, 2001 on the basis of the status of information available at the time, so the case reports described above have not come unexpectedly, and no fundamentally new situation has been created. As a precaution, preventative measures for minimizing the risk had been taken.

A basis assumption for this report was that new infections from the food chain have meanwhile been effectively stopped. As an additional measure, the exclusion of transfusion recipients from donating blood was considered in order to break a hypothetical chain of further spread and possible perpetuation of vCJD by blood products. Such exclusion was also introduced in other countries (UK, The Netherlands, Switzerland, and France as early as 1998 under the assumption of viral transmissions). Such a model calculation using pessimistic assumptions as the basis, however, shows that in taking into account demographic structures, an exclusion of transfusion recipients would not essentially modify the epidemiological course of the disease. Even an effect in the meaning of preventing isolated cases would be minimal at best. On the other hand, the loss of a significant number of blood donors would have a negative effect on the availability of blood, thus necessitating major efforts in motivating new donors. Therefore, introducing such exclusion is not recommended.

This observation and the results from a serial investigation of tonsil tissue in the UK, two of them found to be homozygous V/V at codon 129, could indicate that there is a considerable number of persons infected with the pathogen who have not developed vCJD or in whom its manifestation is delayed. According to current knowledge, it is not possible to judge whether infectivity is present in the blood of these persons, and if so, at what time and to what extent. To be on the safe side, the worst case scenario of infectivity should be used as a basis. The model calculation in this report (see Appendix D) takes these considerations into account. This means that decisions on measures to be taken should not be based exclusively on the number of vCJD cases that have become visible and the development of the number of such cases forecast on the basis of these cases.
The secondary route of infection by blood could largely be stopped as soon as a suitable screening test becomes available. No such test is currently available nor is its availability foreseeable. Providing resources for developing suitable test methods should therefore be treated with high priority.

A possible transmission of the vCJD pathogen by plasma products still cannot be entirely ruled out, but seems unlikely since various experimental systems have shown that prions are largely removed during the manufacture of these blood products. Examining the effectiveness of these steps, however, should be continued in a product-oriented manner. A Note for Guidance was published in 2004 by the European Medicines Agency (EMEA) for this purpose [2].
Statements on Epidemiology

The Occurrence of Bovine Spongiform Encephalopathy

Bovine Spongiform Encephalopathy in Cattle

Europe

The feeding of ruminant material to cattle has most probably caused the occurrence of bovine spongiform encephalopathy (BSE), a disease of cattle that was first diagnosed in the UK in 1986 [3]. Technological changes (pressure and temperature conditions) in the manufacture of meat and bone meal and other products are considered to be the cause for the occurrence of BSE in the UK beginning in 1985. Due to these changes, the inactivation of the BSE pathogen was no longer sufficiently effective [4]. This assumption is confirmed by the course of the epidemic in the UK where a decline in the number of cases was observed during the mid-1990s with a time lag representing the incubation time of 4–5 years for BSE following the ban on feeding meat and bone meal and the regulations on the disposal of BSE-infected animal carcases [5] (table 1). While in the first few years it was assumed that there was only one strain of BSE in cattle, different authors have described atypical BSE cases in the past few years [6–8]. These cases that do not represent a uniform strain are characterized by an altered molecular weight of the accumulated pathological prion protein (PrPSc), a different anatomical distribution pattern of the pathological changes and the PrPSc deposits, and partly by the occurrence of amyloid plaques. All cases of atypical BSE described so far have been found in animals older than 8 years. The cases described in France show a biochemical similarity with the cases of scrapie in sheep. Therefore, the authors discuss the possibility that these might be scrapie infections in cattle.

Through animal trade and trade of feeding stuff components produced from animal carcases and slaughtering by-products (bone meal, fats for milk replacers, grieves, etc.), BSE spread from the UK to other European countries and countries outside Europe (e.g. Canada, Japan, Israel). First Ireland (1989), then Switzerland (1990) and France (1991) reported cases of BSE. During the mid-1990s, Portugal (1994), The Netherlands (1997), Belgium (1997), Luxemburg (1997), and Liechtenstein (1998) followed with their own cases. Toward the end of the 1990s, it became clear that almost all countries with extensive exchange of goods within the European single market in the previous decade were affected by BSE. It was therefore not surprising that BSE was diagnosed in some cattle of Denmark, Germany, and Spain in the year 2000 and also in Austria, the Czech Republic, Finland, Greece, Italy, Slovakia, and Slovenia in 2001. Since 2002, BSE has also been diagnosed in Polish cattle. Cases of BSE in cattle imported from the UK were reported as early as the early 1990s by several European countries (Portugal 1990, Germany 1992, Denmark 1992, Italy 1994). In addition to animal trade and trade with animal products, however, nationally internal factors influenced the occurrence and spread of BSE. Since by the 1980s most EU member states had changed their animal body disposal methods and processed side products from abattoirs without the removal of specified risk materials (SRM), all under pressure and temperature conditions that were not sufficient for the inactivation of the BSE pathogen, this pathogen was continuously spread, thus increasing the number of BSE cases. Moreover, only passive monitoring systems based on the reporting of clinical symptoms were in place, BSE rapid tests were not yet available.

Two BSE cases have so far occurred in the USA of which one animal had been imported from Canada.

Specified Risk Materials

2000). Since the spread of the BSE crisis in Europe, the definition of SRM has been adapted several times (table 2). According to the latest amendment, the tissues designated as specified risk materials must be subjected to safe removal, and must not enter the food chain. The following tissues are designated as specified risk materials: ‘the skull excluding the mandible and including the brain and eyes, the vertebral column excluding the vertebrae of the tail, the spinous and transverse processes of the cervical, thoracic and lumbar vertebrae and the wings of the sacrum, but including the dorsal root ganglia, and the spinal cord of bovines aged over 12 months, and the tonsils, the intestines from the duodenum to the rectum and the mesentery of bovines of all ages; the skull including the brain and eyes, the tonsils and the spinal cord of ovine and caprine animals aged over 12 months or which have a permanent incisor erupted through the gum, and the spleen of ovine and caprine animals of all ages.’

Because of the significant decrease in the number of BSE cases in the EU, the age limit for the collection and safe removal of SRM for the spinal cord of bovines was raised to 24 months (table 2), and a raise of the test age is being discussed (see the ‘BSE road map’ for more details: http://europa.eu.int/comm/food/food/biosafety/bse/roadmap_en.pdf).

**Geographical BSE Risk and BSE Status Categories**

The SSC has developed a procedure by which the geographical BSE risk (GBR) in a member state or non-European country can be evaluated. In its opinion (Final Opinion of the Scientific Steering Committee on the Geographical Risk of Bovine Spongiform Encephalopathy (GBR). Adopted on 6 July 2000), published in July 2000, it laid down the following criteria for classifying one of four risk levels:

- structure and dynamics of the bovine population,
- BSE surveillance,
- cullings in connection with BSE cases,
- imports of bovine animals and meat and bone meal (MBM),
- feeding,
- ban on the feeding of meat and bone meal (MBM bans),
- regulations concerning specified risk material (SRM bans),
- removal of animal carcasses.

The risk levels are defined as presented in table 3. Classification of EU member states into these BSE risk levels was determined and published as well. At that point in time (2000), Argentina, Australia, Chile, Norway, New Zealand, and Paraguay were classified as GBR level I, Austria, Finland, Sweden, Canada, and the USA as GBR level II, whereas the UK and Portugal were classified as GBR level IV. All other countries, including Germany, were classified as GBR level III. Germany's classification as GBR level III caused heated discussions in Germany since, up to that time, the country had been considered to be absolutely BSE free. In actuality, all countries rated into BSE level III indeed identified BSE cases in their own countries within the following months.

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<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Switzerland</td>
<td>0</td>
<td>2</td>
<td>8</td>
<td>15</td>
<td>29</td>
<td>64</td>
<td>68</td>
<td>45</td>
<td>38</td>
<td>14</td>
<td>50</td>
<td>33</td>
<td>42</td>
<td>24</td>
<td>21</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>UK</td>
<td>7,228</td>
<td>14,407</td>
<td>25,359</td>
<td>37,280</td>
<td>35,090</td>
<td>24,438</td>
<td>14,562</td>
<td>8,149</td>
<td>4,393</td>
<td>3,235</td>
<td>2,301</td>
<td>1,443</td>
<td>1,202</td>
<td>1,144</td>
<td>611</td>
<td>343</td>
<td>151</td>
</tr>
</tbody>
</table>


*Source and information on up-to-date statistics: Office International des Epizooties, as of 9 January 2006 (www.oie.int).

*Data for 2005 still incomplete.

*Cases in imported animals.
Based on this SSC classification, regulation (EC) No 999/2001 of the European Parliament and the Council of 22 May 2001 (Official Journal of the European Communities of 31 May 2001, L147, p. 1) which laid down rules on prevention, control and eradication of certain transmissible spongiform encephalopathies classified the member states and third countries into five BSE status categories. The classification in status categories was based on criteria similar to those of the SSC. However, in this context the number of diagnosed BSE cases served as an important additional factor. Consequently, other points of combating BSE laid down in this EU regulation refer to the status category of the appropriate country, such as the required extent of the safe retrieval and removal of SRM.

Since 2001, the GBR was assessed in compliance with the above-described criteria for various other countries, e.g. candidate countries for accession to the EU. Almost all countries were classified as GBR level III, since, although no BSE case occurred in these countries, monitoring had been carried out with too little intensity to guarantee satisfactory statistical safety. A number of countries evaluated in 2000, too, were later re-evaluated, which led to the

<table>
<thead>
<tr>
<th>Directive/Regulation</th>
<th>Definition SRM Bovine Animals</th>
<th>Definition SRM Ovine Animals</th>
</tr>
</thead>
<tbody>
<tr>
<td>1997/534/EC of July 30, 1997</td>
<td>the skull including the brain and eyes, the tonsils, and the spinal cord of bovine animals aged over 12 months</td>
<td>the skull including the brain and eyes, the tonsils and the spinal cord of ovine and caprine animals aged over 12 months that have a permanent incisor erupted through the gum, and the spleen of ovine and caprine animals of all ages</td>
</tr>
<tr>
<td>2000/418/EC of June 29, 2000</td>
<td>the skull including the brain and eyes, the tonsils, and the spinal cord of bovine animals aged over 12 months</td>
<td>the skull including the brain and eyes, the tonsils and the spinal cord of ovine and caprine animals aged over 12 months that have a permanent incisor erupted through the gum, and the spleen of ovine and caprine animals of all ages</td>
</tr>
<tr>
<td>2001/2/EC of December 27, 2000</td>
<td>the skull including the brain and eyes, the tonsils, the spinal cord of bovine animals aged over 12 months, and the intestines from the duodenum to the rectum of bovine animals of all ages</td>
<td>the skull including the brain and eyes, the tonsils and the spinal cord of ovine and caprine animals aged over 12 months that have a permanent incisor erupted through the gum, and the spleen of ovine and caprine animals of all ages</td>
</tr>
<tr>
<td>EC 999/2001 of May 21, 2001</td>
<td>the skull including the brain and eyes, the tonsils and the spinal cord of bovine animals aged over 12 months, and the intestines from the duodenum to the rectum of bovine animals of all ages</td>
<td>the skull including the brain and eyes, the tonsils and the spinal cord of ovine and caprine animals aged over 12 months that have a permanent incisor erupted through the gum, and the spleen of ovine and caprine animals of all ages</td>
</tr>
<tr>
<td>EC 270/2002 of February 14, 2002</td>
<td>the skull including the brain and eyes, the tonsils and the spinal cord, excluding the vertebrae of the tail, but including the dorsal root ganglia, and the spinal cord of bovine animals aged over 12 months, and the intestines from the duodenum to the rectum and the mesentery of bovine animals of all ages</td>
<td>the skull including the brain and eyes, the tonsils and the spinal cord of ovine and caprine animals aged over 12 months that have a permanent incisor erupted through the gum, and the spleen of ovine and caprine animals of all ages</td>
</tr>
<tr>
<td>EC 1139/2003 of June 27, 2003</td>
<td>the skull excluding the mandible and including the brain and eyes, the vertebral column excluding the vertebrae of the tail, the transverse processes of the lumbar and thoracic vertebrae and the wings of the sacrum, but including dorsal root ganglia, and the spinal cord of bovine animals aged over 12 months, and the tonsils, the intestines from the duodenum to the rectum and the mesentery of bovine animals of all ages</td>
<td>the skull including the brain and eyes, the tonsils and the spinal cord of ovine and caprine animals aged over 12 months that have a permanent incisor erupted through the gum, and the spleen of ovine and caprine animals of all ages</td>
</tr>
<tr>
<td>EC 1492/2004 of August 23, 2004</td>
<td>The skull excluding the mandible and including the brain and eyes, the vertebral column excluding the vertebrae of the tail, the spinous and transverse processes of the cervical, thoracic and lumbar vertebrae and the median sacral crest and wings of the sacrum, but including the dorsal root ganglia, and the spinal cord of bovine animals aged over 12 months, and the tonsils, the intestines from the duodenum to the rectum and the mesentery of bovine animals of all ages</td>
<td>the skull including the brain and eyes, the tonsils and the spinal cord of ovine and caprine animals aged over 12 months that have a permanent incisor erupted through the gum, and the spleen of ovine and caprine animals of all ages</td>
</tr>
<tr>
<td>EC 1974/2005 of December 2, 2005</td>
<td>the skull excluding the mandible and including the brain and eyes, and the spinal cord of bovine animals aged over 12 months, the vertebral column excluding the vertebrae of the tail, the spinous and transverse processes of the cervical, thoracic and lumbar vertebrae and the median sacral crest and wings of the sacrum, but including the dorsal root ganglia of bovine animals aged over 24 months, and the tonsils, the intestines from the duodenum to the rectum and the mesentery of bovine animals of all ages</td>
<td>the skull including the brain and eyes, the tonsils and the spinal cord of ovine and caprine animals aged over 12 months that have a permanent incisor erupted through the gum, and the spleen of ovine and caprine animals of all ages</td>
</tr>
</tbody>
</table>
classification of Austria, Canada, USA, Mexico and South Africa to GBR level III. In March 2003, Canada’s second BSE case was discovered (the first case was diagnosed in 1993), and in June 2005, the first BSE case was confirmed in the USA. The results and opinions of the SSC and the European Food Safety Authority (EFSA) can be found in http://europa.eu.int/comm/food/fe/sc/ssc/outcome_en.html and http://www.efsa.eu.int, respectively.

Following the steady decrease of BSE cases in the UK in the past few years, the number of BSE cases reported per 1 million bovine animals older than 30 months has fallen below 1,000 enabling a re-evaluation of the UK as BSE risk status III according to Regulation (EC) 999/2001. The appropriate application was given a favorable opinion by the EFSA, and, based on the BSE risk in the UK now classified as moderate, it was suggested that the UK be classified as BSE risk status III. A change in the BSE risk status represents a significant relief for the UK regarding international trade of bovines and bovine animal products.

Germany
Passive BSE surveillance has been performed in Germany for years, i.e. all bovine animals that died or became clinically sick due to disorders of the central nervous system and were suspected to have suffered from BSE were examined. The brains of such animals were subjected to histopathological examination, and any abnormal results were also examined for plaques of PrPSc by immunohistochemical examination and/or scrapie associated fibril (SAF) extraction with subsequent immunoblot. These examinations did not reveal any BSE cases in German cattle.

The first BSE rapid test, the Prionics Check Western blot developed by Prionics, Switzerland, was available in the mid-1999. Even though the test had not yet been approved European-wide or on a national basis, it was already used in some European countries. A series of tests was carried out in North-Rhine Westphalia, Germany, from March to May 1999 in which 5,000 beef cattle were examined for BSE using this test. All these animals showed negative results, reinforcing the hope of a BSE-free Germany.

In preparation for the transposing of Commission Decision 2000/374/EC, which established random BSE monitoring of bovine animals, a few voluntary BSE examinations were carried out in cattle samples starting in mid-November of 2000. These examinations revealed the first indigenous German BSE case in Schleswig-Holstein confirmed by the national reference laboratory on November 26, 2000. This was followed by the introduction of such examinations throughout Germany within a short period of time. After the extensive introduction of BSE rapid tests in December 2000 for all slaughtered cattle as well as for fallen stock (first over 30, then over 24 months old), 390 BSE cases were identified in the following years (reference date January 16, 2006) (table 4). The number of cases reported annually is steadily declining although a slight increase was observed from 2003 to 2004. Altogether, these data indicate that the BSE ‘epidemic’ in Germany may have already exceeded its peak before the first case was even diagnosed. Simultaneous to the introduction of the BSE rapid test, a total ban on feeding protein-containing products and fats derived from warm-blooded land animals to ruminants throughout Europe was imposed in the year 2000. In Germany, this ban was extended to the feeding of all productive livestock as defined in the Futtermittelgesetz (Act on Feeding Stuffs).

While during the first 2 years of BSE monitoring in Germany the disease was predominantly diagnosed in animals born in 1995 and 1996, BSE has been increasingly identified in animals born in later years (particularly in 1998/99) since 2004. This suggests that after a significant entry of BSE infectivity into the feeding stuff chain in 1995/1996, a reduction must have occurred, followed by a second increase in the pathogen content around 1998/1999. It is still unknown what caused these two BSE waves. Up to late 2004, BSE was diagnosed in 10 bovine animals born in 2000. Then, in April 2005, BSE was diagnosed for the first time in a bovine born in May 2001, i.e. after the implementation of the total feed ban of August 1996 (so-called BARB-BSE cases) were diagnosed up to April 2005 (source DEFRA statistics). There are two explanations for this phenomenon which must be considered as the cause for the occurrence of such cases, individually or together:

i) The routes of infection have not yet been fully identified, and a transmission of the BSE pathogen cannot be excluded 100% despite a strict adherence to the feed ban.

Table 3. GBR BSE risk levels

<table>
<thead>
<tr>
<th>GBR level</th>
<th>Presence of one or more cattle clinically or preclinically infected with the BSE agent in a geographical region/country</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>highly unlikely</td>
</tr>
<tr>
<td>II</td>
<td>unlikely but not excluded</td>
</tr>
<tr>
<td>III</td>
<td>likely but not confirmed or confirmed, at a lower level</td>
</tr>
<tr>
<td>IV</td>
<td>confirmed, at a higher level</td>
</tr>
</tbody>
</table>

Table 4. Number of confirmed BSE cases in Germany per year

<table>
<thead>
<tr>
<th>Year</th>
<th>Number of BSE cases</th>
</tr>
</thead>
<tbody>
<tr>
<td>2000 (as from November)</td>
<td>7</td>
</tr>
<tr>
<td>2001</td>
<td>125</td>
</tr>
<tr>
<td>2002</td>
<td>106</td>
</tr>
<tr>
<td>2003</td>
<td>54</td>
</tr>
<tr>
<td>2004</td>
<td>65</td>
</tr>
<tr>
<td>2005</td>
<td>32</td>
</tr>
</tbody>
</table>
The theoretical risk of transmission of the BSE pathogen to small ruminants has been scientifically discussed for some time. This concern resulted in the introduction of an active transmissible spongiform encephalopathy (TSE) surveillance of these species by means of a rapid BSE test pursuant to Regulation (EC) 999/2001. This regulation laid down the number of samples to be taken on the basis of the respective number of ovine animals for each member state. Thus, 60,000 slaughtered animals and 10,000 risk animals (slaughtered for emergency reasons or dead from other causes) over 18 months of age were to be tested in Germany. However, after it was found that the figure of 60,000 slaughtered animals could not be reached, the sample size was corrected to 10,000 slaughtered animals and 10,000 risk animals as from 2004. After the introduction of this intensive monitoring, the number of reported TSE cases in small ruminants markedly increased in nearly all member states. In Germany 0–3 cases of scrapie had been diagnosed for many years; the figure has risen to 31–119 individual animals with altogether 68 outbreaks of classical and atypical scrapie per year since 2002. In some cases, the disease could be detected in up to 56 animals of the same herd. In Germany, no TSE infection has been diagnosed thus far in any of the 12,000 goats tested since the beginning of the more intensive monitoring. Regulation (EC) 999/2001 also laid down that each TSE case in small ruminants was to be tested by means of biochemical methods or animal experiments (“strain typing”). This measure serves to guarantee that a possible BSE infection would not remain undetected in these small ruminant species. Since the animal experimental methods used up to now mainly for scientific interest [12, 13] are very time-consuming and costly, the samples are usually first tested by means of biochemical methods (analysis of the molecular weight, the glycosylation profile, and the antibody binding affinity of the accumulated pathological prion protein) [14–18]. So far, evidence of BSE infection in sheep has not been found in any of the 37 classical scrapie outbreaks in Germany [18], nor during the relevant tests performed in other member states. The cases of atypical scrapie were excluded from the strain typing since this TSE type is clearly distinct from BSE [19]. More recent tests in France, however, gave clear evidence of a BSE infection. In this animal, a TSE infection was reported within the active surveillance of 2002. Subsequent strain typing tests gave evidence for a BSE infection in this animal. This first evidence that the BSE pathogen can cross the species barrier between cattle and small ruminants gave rise to special concern in expert circles for the following reasons: In small ruminants, the TSE pathogenesis clearly differs from that in cattle. In cattle, pathologic prion protein and BSE infectivity remain strictly limited to the central nervous system and only become detectable immediately before the occurrence of clinical symptoms [20]. The combination of rapid testing of all beef cattle above a certain age (after 30 months in the EU, after 24 months in Germany), in combination with the safe removal of SRM, thus presents an effective consumer protection measure. The situation is different in sheep where the pathogen can be detected in various organ systems very soon after the infection, above all in the nervous and lymphatic systems [21–23]. It is therefore well possible that an animal testing negative in the rapid test of the brain stem has already accumulated disease-related prion protein and infectivity in other organs. Since, however, TSE pathogenesis in sheep depends on various factors, e.g. the PrPSc genotype of the affected animal and the pathogen stem, no uniform testing concept can be determined for this animal species that would guarantee TSE detection at the first possible point in time after infection. BSE infection in small ruminants thus presents a potentially increased risk for the consumer compared with the occurrence of the same disease in cattle.

The Occurrence of vCJD

A new variant of Creutzfeldt-Jakob Disease (vCJD) was described in the UK in 1996. This variant can be distinguished from the classical forms of the disease, both by its clinical and neuropathological characteristics [24–26], 162 probable and confirmed cases as of August 7, 2006 (www.cjd.ed.ac.uk) have been diagnosed in the UK. 156 patients have died. In 112 cases, the diagnosis was neuropathologically confirmed. As of July 28, 2006, 20 cases have been diagnosed in France out of these, 3 in 2004, and 6 in 2005 (www.invs.sante.fr/surveillance), 4 in Ireland, 2 in USA, 2 in The Netherlands, and 1 case each in Canada, Italy, Japan, Saudi-Arabia, Portugal, and Spain. Thus, the vast majority of all vCJD cases have occurred in the UK (83%). Two of the Irish cases, the two cases in the USA, and those occurring in Canada, France and Japan had spent a long time in the UK (between 1 month and 16 years) and may have contracted the disease during their stay there. One Chinese patient who died in Hong Kong had stayed in the UK for several years and is included in the UK cases (table 5). The number of vCJD deaths in the UK reached its peak in 2000 with 28 cases; then, the number of deaths due to vCJD dropped sharply up to 2005. This development currently supports the hope that the epidemic has surpassed its peak in the UK. This assumption, however, is still unsafe due to the lack of knowledge about the disease, duration of the incubation period, and frequency of manifestation dependent on the genotype at codon 129 of the prion protein gene. In France, however, the number of probable and confirmed vCJD cases had reached a total of 20 as of July 2006 and has not shown any decline. Rather, more than half of the cases were diagnosed from 2004 up to July 2006. 17 individuals in France have died of vCJD. The number of persons who died of probable or confirmed vCJD in the UK and in France up to 2005 is shown in figure 1. It is assumed today that vCJD is caused by the same pathogen as that causing BSE in cattle. This is based on studies of the geographic occurrence of BSE and vCJD, the biochemical similarity between BSE- and vCJD-associated prion proteins [27, 28], the non-distinguishability of the pathogen during strain typing (incubation periods in different mouse strains, lesion patterns in the brain.
Corresponding human to human transmission, 3 vCJD cases are seriously suspected to have been caused by blood transfusions (cf. ‘Risk of Transmission of vCJD Disease through Blood (Secondary Infections)’, pp 13). These cases were identified with epidemiologically vCJD surveillance throughout the UK, which assessed whether the vCJD patients known had previously donated blood, and determined who had received blood products produced from these donations. 15 of the vCJD patients who were diagnosed up to December 2003 had donated blood. They had donated a total of 55 labile blood products of which 48 had been transfused. At that time 17 living recipients were identified. In late 2003, it was found that one recipient of said blood products had also died of vCJD more than 6 years after the transfusion of a non-leukocyte depleted red blood cell concentrate (RBCC). The donor of this RBCC was healthy at the time of donation, but died of vCJD 3.5 years after the donation [35]. Since the recipient also lived in the UK, thus presenting a risk for contraction of vCJD via the food chain, transmission by transfusion cannot be proved, but is highly likely considering the results of statistics and animal experiments. In 2004, another recipient of donations from deceased vCJD patients was identified who had died of rupturing aortic aneurysm 5 years after transfusion without any signs of neurological disease. Post mortem revealed vCJD-specific prion protein in a neck lymph node and in the spleen, however, not in the brain [36]. A third case was reported in a press release of February 9, 2006 [37]. This individual developed symptoms of vCJD after receiving non-leukocyte depleted RBCC 8 years ago. The donor of the concentrate developed vCJD 20 months after the donation (Eurosurveillance 2006) [38]. There is currently no evidence for transmission of vCJD by transplants or other medicinal products derived from human material, e.g. plasma derivatives, albeit transmission by this route cannot be excluded in principle.

Apart from this, contrary to classical CJD, there are no reports on iatrogenic vCJD infections worldwide. Classical CJD was transmitted worldwide in more than 100 cases by pituitary (growth hormone, follicle stimulating hormone) and dura mater products. In isolated cases, infection by corneal transplantation and by re-used surgical instruments (i.e. electrodes) was reported [39]. The transmission risk was minimized by suitable measures (e.g. replacement of the pituitary extracts by recombinant products, critical selection of dura mater and cornea donors, treatment of the dura mater with sodium hydroxide solution, use of disposable instruments). Contrary to the classical forms of CJD, however, vCJD patients have measurable pathogen content not only in the central nervous system but also in peripheral tissues [40], especially in lymphatic tissues (tonsil, appendix, spleen). It is therefore conceivable, though not yet observed, that, apart from possible infections by blood or blood products, infection is possible in principle by re-use of instruments in general surgery, including flexible endoscopes.

Recommendations for minimizing iatrogenic vCJD transmissions were put forth in April 2002 by the ‘Task Force vCJD’, established at the Robert Koch-Institut in collaboration with the Scientific

Table 5. Patients with vCJD worldwide and duration of stay in the UK

<table>
<thead>
<tr>
<th>Country</th>
<th>Total number of cases (number alive)</th>
<th>Cumulative residence in UK &gt; 6 months during period</th>
</tr>
</thead>
<tbody>
<tr>
<td>UK</td>
<td>162 (6)</td>
<td>162</td>
</tr>
<tr>
<td>France</td>
<td>20 (3)</td>
<td>1*</td>
</tr>
<tr>
<td>Ireland</td>
<td>4 (1)</td>
<td>2</td>
</tr>
<tr>
<td>The Netherlands</td>
<td>2 (1)</td>
<td>0</td>
</tr>
<tr>
<td>USA</td>
<td>2 (0)</td>
<td>2</td>
</tr>
<tr>
<td>Canada</td>
<td>1 (0)</td>
<td>1</td>
</tr>
<tr>
<td>Italy</td>
<td>1 (0)</td>
<td>0</td>
</tr>
<tr>
<td>Japan</td>
<td>1** (0)</td>
<td>0</td>
</tr>
<tr>
<td>Portugal</td>
<td>1 (1)</td>
<td>0</td>
</tr>
<tr>
<td>Saudi Arabia</td>
<td>1 (1)</td>
<td>0</td>
</tr>
<tr>
<td>Spain</td>
<td>1 (0)</td>
<td>0</td>
</tr>
</tbody>
</table>

* The person from France had traveled regularly to UK over more than 10 years since 1987.
** The person from Japan had resided in the UK for 24 days in the period 1980–1996.

Fig. 1. Deaths caused by probable and confirmed vCJD in the UK and France from 1995–2005 (status of the data UK: March 3, 2006, France: February 28, 2006).
Estimation of the Extent of the Spread of vCJD

Mathematical models have been developed to assess the extent of the vCJD epidemic taking into account certain preconditions. As a rule, these models only take into account the uptake of the pathogen via contaminated beef. The estimates require sufficiently reliable information on the relevant parameters, e.g., minimum infectious dose in the event of oral route of transmission, extent of consumption of contaminated beef, distribution of the incubation periods, and information on the susceptibility of the exposed population. These basic parameters are still not safe, despite the number of additional insights obtained, and every model calculation is therefore inevitably fraught with uncertainties. In addition, human to human transmission may add to the occurrence of the epidemic. In this context, infectivity of parenteral administration of the pathogen needs to be known. No estimates have so far been published on the portion of possible vCJD cases that follows this infection route.

Estimation of the vCJD Epidemic in the UK

Models developed to assess the vCJD epidemic in the UK at first assumed that only a portion of the population can contract the disease. This is based on the observation that clinical vCJD has developed only in those individuals who are homozygous M/M at codon 129 of the prion protein gene. Therefore, it was first assumed for the model that only that part of the population homozygous for methionine at codon 129 was susceptible for the disease. This applies to approximately 40% of the Caucasian population (table 6) [44–48].

Regarding possible incubation periods for vCJD, previous vCJD cases were matched with the occurrence of the BSE epidemic. In each model, different incubation periods (up to 60 years) and differing age-related susceptibilities were taken into account. The models could be made more accurate with the actual progression of the epidemic and the results obtained so that the estimated number of future vCJD cases in the UK caused by food of up to several million [49] could be adapted from 136,000 [50] to 7,000 [51]. This variability makes clear the difficulties in assessing the limited state of knowledge.

Currently, there are also still uncertainties in determining the incubation period and the degree of susceptibility of the exposed population, i.e., the frequency of clinical manifestation. Above all, the above mentioned polymorphism at codon 129 of the prion protein gene seems to play a role in individual susceptibility for vCJD. Although all vCJD patients up to now who developed the disease were homozygous M/M in this locus, in the year 2004 a transfusion recipient who was heterozygous M/V at codon 129 was diagnosed with pathological prion in the lymphatic tissue. The donor of the transfused blood component had died of vCJD 18 months after the donation. The recipient died of rupturing aortic aneurysm 5 years after the transfusion without any evidence of a neurodegenerative disorder. It remains unclear whether this patient who was heterozygous at this gene locus would have developed the disease later or would never have developed it.

Identifying pathological prion in lymphatic tissue of this heterozygous transfusion recipient has raised the question of whether individuals not homozygous M/M at codon 129 of the prion protein gene can be infected. Two possibilities are discussed for this group:

i) The disease never manifests itself in these infected patients (subclinical development).

ii) Infected patients develop the disease after a longer incubation period.

The existence of a possible ‘carrier status’ is supported by the fact that in a retrospective serial analysis of appendix and tonsil material in the UK, 3 of 12,674 appendices tested revealed pathological prion [52, 53]. Recently the genotype of two of these individuals who had not developed vCJD at the time of the examination was sequenced (the third sample was not available for analysis) and found to be homozygous V/V at codon 129 of the prion protein gene [54]. Immunohistochemical examinations revealed that the distribution of the prion differed in 2 out of the 3 cases from that found in the lymphatic tissue of vCJD patients. It is currently still unclear whether this presents an indication as to the future outcome of the disease or which role methodical problems play in the evaluation with regard to sensitivity and specificity. In addition, it must be borne in mind that the patients from whom the samples were drawn were not representative of the general population due to age distribution.

If this random sample of histological examinations is used solely as a basis and 100% sensitivity and specificity of the test used is assumed, the estimated prevalence of undetected vCJD infections per 1 million inhabitants in the UK would amount to 235 (range 49–692). This would mean a higher prevalence of vCJD than previously assumed and estimated on the basis of the decreasing figures.

The mathematical models developed for estimating the vCJD epidemic were adapted to these new findings. A wider genetic susceptibility and a possible carrier status were assumed for the disease [1]. The authors assume that, taking into account the remaining uncertainties on the length of the incubation period, the number of clinical vCJD cases by the year 2080 will be 70 (range 10–190) based on

<table>
<thead>
<tr>
<th>Individuals tested</th>
<th>M/M, %</th>
<th>M/V, %</th>
<th>V/V, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patients with sporadic CJD</td>
<td>69–78</td>
<td>12–15</td>
<td>10–16</td>
</tr>
<tr>
<td>Patients with vCJD*</td>
<td>100</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

M = Methionine, V = valine.
*Clinical vCJD cases.
the existing calculation model and assuming a developed model for a carrier status, as opposed to 363 cases (no confidence interval indicated), based on a more pessimistic assumption. If the data published on the examinations of the appendices by Hilton et al., [53] are taken into account in the model, the estimation by Clarke and Ghani [1] is 133 (range 32–3,780) cases. Since there is currently still a marked contradiction between the number of patients who actually have developed clinical vCJD and the results of the appendix survey, studying a greater number of appendices and tonsils is planned in order to obtain a more accurate estimate of the prevalence of pathological prion in lymphatic tissue. Evidence from the most up-to-date model calculation, however, points to a limited number of future primary vCJD cases in the UK. Currently, the models are not designed to perform an estimate of the occurrence of transmission from human to human. The model published by Clarke and Ghani [1] in 2005 also indicates estimates for the number of individuals with subclinical and preclinical infection with the vCJD pathogen. The data on the histological examinations of the appendices were taken into account in this assessment, and 50% sensitivity of the tests used was assumed for subclinical infection. Based on these assumptions, a far greater number of individuals infected but without clinical manifestation (1,130–13,440) can be assumed. The number of these preclinical or subclinical carriers and the question of their infectivity, however, is of considerable importance for possible iatrogenic transmission. It may markedly influence the absolute number of future vCJD cases. It should also be examined whether the disease would persist in the population regardless of the food-associated risks and what measures could be used to prevent this. Besides transmission by the use of blood products, incomplete disinfection of surgical instruments also plays an important role.

**Estimation of the vCJD Epidemic Outside the UK**

The above calculations for estimating future vCJD cases refer to the UK. For countries without or with only a small number of vCJD cases, the estimate is even more uncertain. The decisive fact in estimating risk in this case is the extent of exposure to beef potentially contaminated with BSE. A detailed presentation of the incidence of BSE at the peak of the epidemic in various countries affected as well as the assumed period of exposure to BSE cases in Germany are contained in the report of the Arbeitsgruppe Gesamtstrategie Blutversorgung ange­sichts vCJ (Working Group Overall Blood Supply Strategy with regard to vCJD) from 2001 [55]. This makes clear that the extent of the BSE epidemic in the UK is greater by a multiple of that of other countries, even if differences in the reporting criteria are taken into account. A risk of exposure for countries with no or only few BSE cases must therefore take into account the extent of imports of beef cattle from the UK within the relevant period of time. Figure 2 shows imports of beef from the UK between 1990 and 1995.

No reliable data are available for Germany on the extent of BSE exposure due to German cattle or beef imports. The mathematical models from the UK on estimating the vCJD epidemic were used in Ireland and France, taking into account the actual situation in these countries. In Ireland where 4 cases of vCJD have occurred up to now (2 of them were residents of the UK for a considerable period of time), an estimation was performed on the basis of the model developed in the UK with adaptations for conditions in Ireland [56]. The estimation considers the intake of potentially contaminated meat of Irish cattle, cattle imports from the UK and the consumption of...
British beef, and the consumption of British beef during visits to the UK. This model, too, takes into account only the group of individuals who are homozygous M/M at codon 129 of the prion protein gene. On the basis of the data available, it was estimated that 1–2 (range 0–46) more clinical cases of vCJD would occur in Ireland. Apart from the above limitations, the adapted model is suitable for performing an estimation for countries with few or no cases of vCJD, if the basis data are known.

In France, 20 vCJD cases have been reported so far. In a current model calculation, also based on the epidemiological data from the UK, it was estimated that after 2004, another 33 vCJD cases would occur out of which 12 would occur in 2004 and 2005 [57]. Estimates, linked to the birth year, are shown in table 7.

The model calculation takes into account imports of British beef to France, beef consumption and travel to the UK. The estimate of the case numbers for France has thus decreased by two thirds compared with the previous forecasts from 2000 [58].

| Table 7. Estimated vCJD incidence in France (median (5th and 95th percentile)) [57] |
|---------------------------------|-----------|-----------|-----------|-----------|
| Before 1940                     | 0 (0.1)   | 0 (0.1)   | 0 (0.1)   | 0 (0.0)    |
| 1940–1969                       | 1 (0.4)   | 3 (0.9)   | 4 (0.12)  | 3 (0.9)    |
| After 1969                      | 11 (2.32) | 8 (1.23)  | 0 (0.1)   | 0 (0.0)    |

No case of vCJD has so far been diagnosed in Germany. Since the epidemiological situation in Germany is thus markedly different from that in the UK and, in addition, the extent of BSE exposure to potentially contaminated beef cannot be accurately quantified, no primary data allowing a valid use of models for estimating the incidence of primary vCJD cases in Germany are available. Based on estimates for France and Ireland, where only a few vCJD cases have been diagnosed, it can be assumed – on the basis of the current state of knowledge – that only isolated cases of vCJD will occur in Germany.
Safety of Blood and Blood Products with Regard to vCJD

Risk of Transmission of vCJD through Blood (Secondary Infections)

Various approaches could answer the question of whether blood and blood products constitute a risk of vCJD transmission. On the one hand, data from animal experiments could be helpful. On the other hand, epidemiological and case control studies as well as the observation of individual cases serve as important sources of information.

There is a great number of studies attempting to clarify the possibility of transmission of prions by blood and its components in a great variety of combinations of TSE agents and animal species [47, 59, 60]. Most of the previous results on TSE infectivity in the blood of ‘donor animals’, however, must be interpreted with a number of restrictions. Firstly, the investigational material of the ‘donor animals’ (blood, serum, cells, etc.) was usually given to the ‘experimental animals’ (‘indicator animals’) by i.c. administration. Although this permits a more sensitive detection of the pathogen, it makes comparability with intravenous (i. v.) administration more difficult. Secondly, many experiments were performed with animals infected in a ‘non-natural’ manner which makes the extrapolation of the corresponding results more difficult. In addition, the tests are often set up in such a way that they involve species barriers, which in turn means a decrease in sensitivity.

In view of many contradictory results, only limited statements can be made on the basis of experiments in small rodents:

- In principle, infectivity can be detected in the blood of experimentally infected animals.
- Titers of infectious TSE agent in the blood of artificially infected animals were found to be very low (1–100 IU/ml) in sensitive detection systems. The question arises in some experiments as to what extent the detected infectivity really reflects replicated agent and not just residual inoculum. Concentrations of viruses which are known to be transmissible by blood (HIV, HBV, HCV) can be considerable in the early infection phase (diagnostic window), and can amount to up to 10^6 (HIV), 10^5 (HBV) or 10^8 (HCV) virus genomes/ml blood. Thus, infectious units of viruses can be several orders of magnitude higher compared with TSE agents (see above), since only few virus particles are sufficient to infect a recipient, at least in the case of HBV and HCV.
- In various experiments, i. v. transmissions have led to infection of the indicator animal markedly more rarely when compared with i.c. inoculation. In another experiment mice were infected with either mouse-adapted strains of Gerstmann-Sträussler-Scheinker (GSS), a special familial form of CJD, or vCJD. Infectivity of approximately 20–20 IU/ml was found 17 and 23 weeks after i. c. inoculation in the preclinical and the symptomatic phases, in both theuffy coat of the blood and the plasma [61]. In this experiment, no major difference in effectivity was found between i. v. and i. c. inoculation.

However, transmissibility by blood was already assumed in the report of August 17, 2001 on the basis of the data available at that time. In the following period more evidence accumulated that pointed to a potential transmissibility of prion diseases by blood, particularly on the basis of a publication of a transfusion experiment in sheep [62]. In this experiment, blood marked for transfusion (whole blood or buffy coat) was drawn from sheep in two series of experiments. The sheep had been either orally infected by brain material from BSE-infected cows or had originated from a herd of sheep with particular genetic susceptibility for scrapie. After transfusion of the blood (partly whole blood, partly buffy coat) of ‘donor animals’ that later died of the orally induced ‘BSE’ or scrapie, definite TSE developed in a number of ‘recipient animals’, and the transmitted pathogen could be identified. Transmission of TSE must therefore be stated in this experiment. The only limiting remark that must be made is that the ‘recipient animals’ were sheep particularly sensitive to TSE because of their genetic nature. Unfortunately, the diagnostic detection of prions in the blood of such ‘donor’ or ‘recipient’ animals has not yet been performed.

Formal retrospective epidemiologic studies and case control studies on a possible transmission of human TSEs by blood or blood products did not reveal any evidence of this route of transmission. While a number of viruses (HIV, HCV, HBV) have been transmitted to the recipient group of hemophiliacs to a large extent by not at all or not sufficiently inactivated factor concentrates from infected blood and plasma donors in the past few decades, no case
of a hemophiliac who developed classical CJD has become known [63]. Since this disease can barely be overlooked in this well monitored group of patients and since it is also known that some individuals who later developed CJD had also been blood donors, there is no evidence for a transmission of classical CJD pathogen by blood products.

However, two case reports published in the UK in 2004 played a decisive role in estimating the transmission risk by transfusions in humans. A special monitoring system, the National CJD Surveillance Unit, was established in the UK in 1990, which, among other things, was designed to identify blood donors among vCJD patients and locate their donations. It was also responsible for observing the recipients of these donations and the introducing of appropriate tests after their deaths, which included an autopsy with histopathological identification of the vCJD agent. As a result 48 recipients of blood components of 15 vCJD donors were identified and monitored. This surveillance system has so far identified three cases:

**Case 1:** In 1996, a then 62 year-old patient received a total of 5 RBCC within one operation. One of these concentrates (non-leukocyte-reduced) originated from a donation of a 24-year-old individual who died of confirmed vCJD in the year 2000 (3.5 years after the donation). The recipient developed symptoms toward the end of 2002 (6.5 years after the transfusion), and died of the clinical symptoms of vCJD 13 months later. The diagnosis was confirmed by a postmortem examination [35]. The probability of coincidence, i.e. an infection via the food chain not related with the transfusion, was rated as very low (1 : 15,000 to 1 : 30,000).

**Case 2:** The second case was another elderly patient who received a non-leukocyte-reduced RBCC from a donation of an individual who developed symptoms 18 months later and died of confirmed vCJD in 2001 [36]. The recipient died of a non-related cause (rupturing aortic aneurysm) 5 years after the transfusion without any signs of a neurologic-psychiatric disorder. Within the above-described surveillance, a postmortem was carried out. The vCJD agent was found by histopathologic examination in the spleen and the lymph node, but not in the CNS. While all previously identified cases of patients who developed vCJD were homozygous M/M at codon 129 of the prion protein gene, this case – which, however, showed no CNS disorder clinically and histopathologically – was heterozygous M/V at codon 129.

**Case 3:** A third case in the UK was reported in a press release of February 9, 2006 [37]. It was reported that an individual developed vCJD 8 years after transfusion of a non-leukocyte-reduced RBCC. The transfused individual was hospitalized at the time of the press release. The diagnosis of vCJD was rated as ‘likely’ by the National CJD Surveillance Unit. No details were disclosed concerning this. In addition, no details were given on the status regarding codon 129. The donor had developed vCJD 20 months after the donation. No diagnostic system is available for vCJD in contrast to virus infections by means of which we would be reliably able to prove or exclude transmission in individual cases. Joint consideration of the first two cases, however, does not permit any other conclusion than transmissibility of the vCJD agent by blood transfusion.

The first two cases were associated with red blood cell transfusion. No leukocyte reduction of RBCC was carried out in either case. However, no conclusion may be drawn that such treatment of the components would have prevented transmission. More recent studies [64] confirm that leukocyte filtration would reduce the cellularly bound part of infectivity but would only remove approximately 42% of endogenous (not artificially spiked) infectivity from the blood of scrapie-infected hamsters. Therefore, leukocyte reduction – which in Germany was actually introduced mainly for other reasons – can be regarded as a useful measure but not as a reliable protection against vCJD transmission.

In the second case (see above), the agent was obviously transmitted. However, an involvement of the CNS was not detectable – neither clinically nor histopathologically. The infection was therefore subclinical at the time of death. Whether vCJD would have developed in this case must remain open.

The decisive factor for estimating the order of magnitude of the general risk of becoming infected with vCJD by transfusion is the number of individuals of a given population who carry the agent in their blood and are able to transmit the pathogen by blood donations. Previous estimates are based on the epidemiologic data on the occurrence of vCJD. It is therefore certainly important whether a relevant number of infected but non-diseased individuals can be expected in addition to the forecast number of vCJD patients. For the time being, the question of whether blood donations of individuals who carry the pathogen but do not have the disease would be infectious cannot be answered. However, the fact that prions were detected in the spleen and lymph nodes of the second case will make it seem advisable to assume infectivity of such donations.

A study showing evidence of prions in three cases during a series of histological tests in 12,674 tonsils [53] is another indication of higher frequency of infection. If this result was representative for the British population, we would have to expect subclinical cases, i.e. individuals who have not or not yet developed vCJD, with a frequency of 1 in 4,000. Taken together the second case of transfusion-transmitted vCJD, which was heterozygous at codon 129, and the fact that two of the appendices, which revealed pathological prion had been tested homoygous V/V at codon 129 of the prion protein gene, one would now hypothesize that all individuals but not only those homoygous M/M, who represent 40% of the population, are susceptible to the vCJD agents. An extrapolation may show that a higher number (maximally double) of infected individuals must be assumed than has been assumed up to now from the epidemiology of vCJD.

This means that decisions on precautionary measures cannot be exclusively based on the previously visible development of the number of typical vCJD cases. We should rather reckon on an additional number of infected individuals who remain permanently subclinical and whose blood donations could be infectious.

As long as a test for vCJD does not exist, clarification and assessment of cases of suspected transmission would be difficult and only possible to a limited extent. In the event of a suspected case of vCJD, this applies both if it was found that the affected individual...
The amount of infectivity in blood is assumed as an estimated value on the basis of data from animal experiments. Analogously to the procedure developed by the French authority AFSSAPS [67], the first version of this report, too, assumed that as the worst case scenario infectivity in whole blood is maximally 100 IU/ml i.c. For i.v. inoculation, efficiency of transmission may be lower. The assumed worst case scenario has been 10 IU/ml i.v. However, in transmission experiments with pri­mates, survival rates after i.v. and i.c. inoculation were similar [30, 68], which would indicate similar efficiency of transmission. In addition, in more recent and more comprehensive studies, 13.6 IU/ml i.c. were measured in the blood of hamsters experimentally infected with scrapie [69], and approximately 20 IU/ml i.c. in the plasma of mice infected with adapted vCJD or GSS pathogens [61, 70]. AFSSAPS assumes an infectivity of 20 IU/ml i.v. in the blood based on this new data. For leukocyte-depleted plasma, a reduction of the pathogen concentration of 50% is assumed, thus 10 IU/ml i.v. in leukocyte-depleted plasma instead of previously 1 IU/ml i.v. plasma [67]. A study performed for the British Health Ministry (DNV-Consulting, 2003) [71] assumes a pathogen content of 10 IU/ml i.c. in the plasma and a 5-fold reduction in the case of i.v. inoculation, thus 2 IU/ml i.v.

- This estimate is extrapolated to vCJD cases even though no infectivity has been found in the blood so far [72, 73].
- There are no accurate results from times at which infectivity could be present in the blood of individuals, i.e. during the incubation period and during the course of disease. In the first probable case of vCJD transmission in the UK [35], the erythrocyte concentrate in question had been manufactured 3.5 years before the donor developed the disease. The second case involved a donation of an individual who developed symptoms 18 months later and died of confirmed vCJD in 2001 [36].
- The form of infectious prions (association with cells, monomers, multimers, aggregates, fibrils) in the blood of ‘naturally’ infected creatures is unknown. Based on appropriate animal experiments [74], it was assumed that 90% of the infectivity of whole blood would be present in the cellular fraction and 10% in the plasma. More recent studies [61], however, point to an approximately equal distribution of the amount of pathogen in the plasma and in the leukocyte fraction.
- In 2004, two cases were reported that provided evidence of a transmission of the vCJD pathogen by blood components. In one case, the recipient died 6 years after transfusion of an erythrocyte concentrate originating from a donor who later died of vCJD [35]. In the other case, the vCJD pathogen was identified in a patient who did not have vCJD 5 years after transfusion of an erythrocyte concentrate. The blood donation concerned originated from a donor who later died of vCJD [36]. A third suspected case of transmission in the UK has been reported recently (cf. ‘The Occurrence of vCJD’, pp 7; ‘Risk of Transmission of vCJD through Blood (Secondary Infections)’, pp 13).

Blood Components for Transfusion, Leukocyte Depletion

Leukocyte depletion became compulsory as a precautionary measure against a possible transmission of vCJD pathogens by blood components in various countries, including Germany (cf. ‘Exclusion of Certain Categories of Persons from Donating Blood’, pp 21). Treatment of whole blood (2.5×10⁶ leukocytes/ml) results in a reduction of leukocytes by 3–4 log steps with residual numbers of up to 10⁷ leukocytes per blood component. A theoretical assessment was made in the previous report on the effectivity of leukocyte reduction. In doing so, it was assumed that 90% of the infectivity would be present in the leukocyte fraction [74]. However, a more recent study [61] shows an approximately equal distribution of infectivity in plasma and in the leukocyte fraction (buffy coat) in mice. An experimental study has now been conducted to examine the capacity of leukocyte depletion to remove the TSE pathogen in an experiment with 500 ml blood of scrapie-infected hamsters [64]. 42% of the pathogen were removed during leukocyte depletion, i.e., the concentration was reduced from 13.1 IU₉₀/ml in whole blood to 7.6 IU₉₀/ml. Since the actual concentration of the pathogen in human blood is unknown, it is difficult to assess to what extent this reduction of the pathogen represents a gain in safety.

RBCC

In Germany, RBCC predominantly originate from whole blood donations. Before leukocyte depletion was enforced in Germany (October 2001), the so-called ‘buffy coat-free’ RBCC were the standard preparations which, according to the applicable recommendations (guidelines governing the collection of blood and blood components and the use of blood products (hemotherapy), Bundesärztekammer (German Medical Association) and Paul-Ehrlich-Institut, 2005) [75], and the Council of Europe recommendations may contain in an average volume of 250 ml up to 1.2×10⁷ leukocytes.
Even after the leukocyte depletion has become mandatory, a potential transmission risk of RBCC must be assumed on the basis of the above described assessment.

**Platelet Concentrates**

80% of the platelet concentrates in Germany are manufactured from whole blood donations (e.g.uffy coat, usually pooled from 4–6 donations) and approximately 20% from apheresis. Titors of approximately 10 IU/ml [61] were measured in the thrombocyte fraction of mice. Therefore, residual infectivity must be assumed even after 42% reduction of the pathogen concentration by leukocyte depletion of the whole blood. On the other hand, leukocyte depletion is well justified, especially in platelet concentrates, because of the well-known advantages regarding adverse immunological effects.

A preference for apheresis platelet concentrates is not justified at the present state-of-the-art since the assessment of a residual infection is difficult in apheresis. A relatively high blood volume is processed here while the behavior of vCJD infectivity in the apheresis system is very difficult to predict.

**Plasma for Transfusion (Fresh Frozen Plasma; FFP)**

In Germany, quarantined plasma (Q-P) and solvent/detergent treated plasma (SD-P) are available. The market share of SD-P is approximately 10%. SD-P is manufactured by pooling approximately 700–1,200 individual donations. The volume for a unit of Q-P in Germany is approximately 230–280 ml, and for SD-P it is 200 ml. Based on the assumption made in the previous report, the content in cell-free plasma had been estimated to be 1 IU/ml i. v. Thus, 250 ml of Q-P would contain 250 IU i. v. cell-free plasma.

Two calculations have been made for SD-P:

- a) Based on the assumption that infectivity is distributed homogeneously in the pool, the following result would be obtained: 200 ml individual plasma containing approximately 200 IU i. v. (assuming residual cells are neglected, see above) would be contained in a pool (assuming a relatively low number of approximately 500 donations); this would result in the dilution in the again separated SD-P to 0.4 IU i. v. per plasma bag.

- b) Based on the assumption that infectivity is in principle not evenly distributed in units < 1 IU i. v., the situation would be different: An infectious donation would contain 200 IU i. v. in 500 donations which could be distributed to a maximum of 200 plasma bags, i.e. 200 of 500 SD-P would be infectious. Assuming 1 out of 120,000 donations were infectious (pessimistic assessment at AFSSAPS, 2000) the risk would be 1 out of 240 SD-P batches, if a pool size of 500 donations (low assumption) was assumed. The risk of an infectious SD-P would thus be approximately 1 in 240 × 2.5, i.e. 1 in 600, and would be less favorable compared with 1:120,000 for Q-P from an individual donation according to this calculation.

Based on the assumption of 10 IU/ml i. v. instead of previously 1 IU/ml i. v. in the contaminated plasma donation [67], the risk becomes higher to the disadvantage of the pooled plasma. Based on this assumption, the above calculation (a) for a pool of 500 donations and one donation containing 2,000 IU would result in an average burden of 4 IU in all plasma bags of a batch. If it was assumed that infectivity in principle is not distributed in units < 1 IU i. v. (b), the situation would also be different: an infectious donation would contain 2,000 IU i. v. in 500 donations so that all 500 plasma bags from a pool of SD-P would be infectious. However, since these calculations contain many unknowns (e.g. reduction effects) and are based on unproven hypotheses (cf. 'General Aspects', pp 15), no recommendations can be given here as to the preferred type of plasma.

Another question is whether possible infectivity in the plasma can be reduced by further measures. There have been considerations of making plasma cell-free to the greatest possible extent and also to remove cell fragments by membrane filtration through a membrane with appropriately small pores. This approach has been pursued in France. No experimental evidence is available on whether this could effectively reduce the infectivity of plasma. Furthermore, it is currently not clear whether such filtration would impair the quality of the plasma (e.g. activation of coagulation factors, neoantigen formation). Therefore, a decision in favor of introducing such membrane filtration seems currently premature.

**Industrial Products from Pool Plasma, Nanofiltration**

The evaluation of individual fractionation and inactivation steps in the manufacture of plasma derivatives (e.g. factor concentrates, immunoglobulins, albumin), regarding its effect on vCJD pathogens in the plasma pool, and the resulting risk for the recipient, is still fraught with uncertainties:

- a) Some assessments are based on the assumption that existing vCJD infectivity can be pushed below a presumably safe threshold dose by means of several dilution and reduction steps. In this context, it has not yet been determined whether a dose at the infectious threshold would have to be administered once or whether several doses ‘below the threshold’ would have a cumulative effect in the recipient causing infection.

- b) Opinions are divided as to whether the size of the fractionation pool plays an important part (analogous with the SD-P):
  - Due to the use of a large pool and possible contamination of the products manufactured from this pool, a large number of recipients could be at risk (cf. ‘Appendix (A) Effect of Pool Size on the Potential Risk of vCJD Transmission’; pp 26). It could be deduced from this risk that very small pools would have to be used in order to limit the risk of infection for a great number of recipients.
  - On the other hand, a freely distributed infectivity (e.g. if prion monomers were present) would be diluted considerably by pooling. Therefore, larger pools could present less risk.

For a reliable assessment of the influence of the pool size, more knowledge would be required on the infective dose in humans, the degree of aggregation of infectivity, its dispersibility, and the pathogen concentrations which can occur in the blood of asymptomatic donors.
Based on the assumption that the pathogen behaves like a virus, calculations about the connection between pool size and transmission risk can be found in ‘Appendix (A) Effect of Pool Size on the Potential Risk of vCJD Transmission’ (pp 26). The calculations can be used to derive the result that if a recipient requires life-long treatment, a reduction of the pool size does not contribute to minimizing the risk.

The current situation in plasma fractionation is relatively heterogeneous for products on the German market due to different manufacturers, different countries of origin of the starting plasma, various import products and a great variability of the manufacturing methods.

**Effectivity of the Plasma Fractionation Steps**

Usually, infectious material from brains of scrapie- or BSE-infected hamsters or mice is used to assess the capacity of process steps to remove the vCJD pathogen. The question that arises is to what extent such material is representative of the potential vCJD pathogen derived from human blood. In a comparative study, no differences were observed between the removal of PrPSc from the brain of humans who had developed vCJD, sCJD, or GSS and of PrPSc from the brain of scrapie-infected hamsters [76]. So far, no major differences of pathogen reduction have been reported when different detection methods were used (PrPSc detection versus bioassay) on the pathogen reduction measured [69, 77, 78]. However, the method of preparation of the infectious material from brain can influence pathogen removal. Highly purified PrPSc can aggregate into high molecular fibrils, which behaves differently to dispersed brain material or infectivity in the microsomal fraction [79]. The degree of aggregation is particularly important for pathogen retention in nanofiltration [80] (cf. ‘Appendix (B) Nanofiltration’; pp 27), and precipitation and separation by means of centrifugation and depth filtration. It was shown that PrPSc tends to aggregate in the alcoholic production intermediates during plasma fractionation [81, 82]. Despite the above mentioned uncertainties in the interpretation of the experimental data, a reasonably homogeneous picture is revealed for plasma fractionation.

Several publications are available for the conventional purification steps (alcohol fractionation steps) of plasma derivatives [69, 79, 82–86], which all result in the statement that the pathogen is removed successively from the albumin and the immunoglobulin fractions. For coagulation factors, however, such a generalization is by far more problematic since individual production processes may differ considerably. This is why the EMEA position paper of June 23, 2004 (EMEA/CPMP/BWP/2879/02) required manufacturers to assess their production methods specifically and to carry out their own experimental trials if suitable published results were not available. PrPSc reductions by at least 4 log steps have been reported so far for factor VIII [77, 85, 87].

**Nanofiltration**

Considerable reduction factors are partly reported by filter manufacturers and plasma fractionators on the effectivity of nanofiltration. However, studies were carried out with differing TSE spiking materials (e.g. fibrillar, detergent-treated material, brain homogenate). The infectious form(s) of the vCJD pathogen is (are) currently still unknown. What the effect of the nanofilters would be on smaller prion aggregates remains open. It is assumed that for prion monomers, no mechanic exclusion by pore size would be given. However, reduction on the basis of other interactions with the filter materials cannot be excluded. The actual benefit of nanofiltration for the removal of vCJD pathogen therefore remains fraught with some uncertainty.

A detailed discussion of nanofiltration can be found in ‘Appendix (B) Nanofiltration’ (pp 27).

**Factor VIII after Nanofiltration**

Until recently, the view prevailed that nanofiltration was not possible with large sensitive molecules such as factor VIII. This option, however, has just been implemented by the French manufacturer LFB (pore sizes 35 nm and 15 nm). Since problems could occur in this case that cannot be assessed in laboratory tests, e.g. the development of neoantigenicity, clinical testing should be discussed before such a product is given the marketing authorization. The change in France was effected without clinical trials. However, no additional adverse effects have so far been observed after the change.

**Recombinant Plasma Products**

It can be said that for plasma products, different manufacturing steps can considerably reduce vCJD infectivity from the starting material, but the extent of this reduction must be further tested and validated. The risk of infectious fractionated plasma products should be markedly lower compared with blood components.

With the current state of knowledge, there is no need to advise against the use of plasma derivatives if the indication is made correctly. In hemophilia treatment, decision between coagulation factor products manufactured from plasma and recombinant coagulation factors must be considered very carefully taking into account the situation of the individual patient. A schematic recommendation cannot be given here.

The assessment of the safety of recombinant products is not the main subject of this report. Therefore, reference is made only briefly to a few aspects. Concerning prion safety, it has to be stated that human plasma derivatives, essentially albumin, may be used as a stabilizer during production of the recombinant products. In the manufacture of recombinant products in eukaryotic cell cultures, materials of bovine origin are sometimes used, so the risk of primary infections with the BSE pathogen must be considered in principle. However, such a theoretical risk is minimized by the appropriate international directives (EMEA, 2003 and 2004) [2, 88], e.g. by purchasing the materials from BSE-free countries. Individual tolerability of different products in the patient and relative frequency of the development of inhibitors must also be considered in the overall assessment of safety. In the past, bottlenecks have existed in the supply of both recombinant coagulation factors and coagulation factors prepared from plasma.
Possible Measures for Reduction the Potential Risk of vCJD Transmission by Blood and Blood Products

Measures for the Optimal Use of Blood Products

Blood products are an essential component of any modern clinical treatment. Being ‘medicinal products from humans’, they cannot be entirely risk-free, despite the great progress in safety. Critical indication and restrictive administration of blood products are therefore essential tools that reduce the residual risk, which is also true for a potential transmission of vCJD by donor blood.

Examples of barely scientifically justifiable differences in the indication of blood transfusion can be found in the Sanguis Study [89, 90]. In the extreme case, the preoperative request for provision of RBCC in cholecystectomy was more than 10 times that of the actually transfused units. Noticeable differences were found in the frequency of transfusion among 43 hospitals participating in the studies from 10 European countries. For hemicolecotomies, the range was between 0 and 79% of the patients; such differences cannot be explained by differences between the patient groups studied. This heterogeneous transfusion practice has not changed significantly in the past few years: a more recent Finnish study thus shows that, contrary to international recommendation, the median of pretransfusional hemoglobin (Hb) values in transurethral prostate resection was 112 g Hb/l [91]. Various authors have stated unanimously that prospective determination of administration criteria and consistent instructions of personnel would lead to a considerable reduction of the consumption of blood components [92, 93].

A possible approach to avoiding potential risks relating to (allo- geneic) blood products prepared from donors would be treatment of patients by autologous blood transfusion. The risk of a new infection with blood-associated pathogens (including vCJD) is excluded in autologous blood treatment. Such autologous blood transfusions, however, can be performed only in elective surgery with a timely, sufficiently and reliably foreseeable transfusion requirement. The Arbeitskreis Blut has currently made a statement on the applicability and the importance of autologous blood treatment [94].

Under German EU presidency in 1999, a meeting was held in Wildbad Kreuth [95] with experts in attendance from the EU member states. During this meeting, an assessment of the current situation concerning the use of the most important blood products was elaborated, and central questions of critically assessed use, quality management, and economic aspects of transfusion medicine were summarized. It would be desirable to continue the initiative of Wildbad Kreuth.

In regards to therapy with blood components and plasma derivatives an interdisciplinary working group of the Bundesärztekammer summarized basic principles for a clinically indicated use of all important blood products with special consideration of the international literature, national and international consensus conferences and clinical experiences, and has made this publication accessible to all centers of transfusion medicine in Germany [96]. At a European level, too, appropriate recommendations were adopted on hemotherapy [97].

There has been increased awareness of this problem in the past few years. An essential contribution for this has been the requirement laid down in the Transfusion Act (Transfusionsgesetz; TFG) of July 1, 1998 for the establishment of a well-functioning quality assurance system for the use of blood products in health care facilities. According to the TFG, these health care facilities must employ appropriately qualified physicians as persons responsible for transfusion and, in addition, transfusion representatives in each clinical unit. For this purpose, the Professional Organization of German Transfusionists (Berufsverband Deutscher Transfusmediziner e. V.) has developed a model quality management handbook that is regularly updated, as are the guidelines and guidances of the Bundesärztekammer.

The requirements for such a quality assurance system were transposed into the hemotherapy guidelines [75] where they were further specified. Of crucial importance will be the way in which these guidelines are implemented and used by hospitals and doctors, efforts of which must not decline.

It is necessary that such efforts are actively encouraged on the part of the top managers of health care facilities and by the health policy makers and that they are recognized by the health care providers who are supposed to finance them. We must demand the further development and implementation of guidelines and guidances and their transposition into well-functioning systems for
quality assurance in the use of blood products in health care facilities as an essential contribution to safety, particularly in an effort to avoid a potential vCJD transmission. The demand for an optimal use of blood and blood products has still not lost its justification. This requirement is undisputed, especially as a safety measure in view of vCJD. Shortly after the first suspected case of clinical transmission was disclosed, the EU Commission called in a ‘Technical Meeting of Blood Experts related to vCJD transmission’ on January 20, 2004 in Luxembourg. One of the statements in the paper elaborated by this meeting reads as follows:

‘There was agreement that optimal use of blood may further reduce the risk of transmission of vCJD by avoiding unnecessary exposure to allogenic blood transfusion. In addition avoiding unnecessary transfusion may improve the availability of blood for transfusion; this in turn may facilitate the introduction by Member States of additional donor deferrals if required.’

### Diagnosing vCJD: Screening Tests

When it comes to diagnosing vCJD infection or the disease itself, a distinction must be made between screening tests on one hand and tests for clinical diagnosis on the other. The development of screening tests for vCJD is being intensively pursued by a number of groups, but so far no concrete success has emerged from any of the various approaches. The principal goal of a screening test is to detect vCJD infections as early as possible before onset of initial symptoms in order to prevent possible further transmissions and, if appropriate, to allow therapeutic measures to be taken in an appropriate time frame.

Clinical diagnosis, in contrast, is carried out on patients who are already displaying symptoms of the disease. In this case it is a question of investigating the suspicion of vCJD or another neurological disease based on various parameters. Definitive diagnosis of vCJD can in principle be carried out in live patients by screening for PrPSc in the tonsils [28, 40]. However, a biopsy that presents such a burden for the patient would only, if at all, be performed in the case of serious suspicion. At present, however, a confirmation of vCJD infection by histological display of the amyloid plaque or detection of the PrPSc in brain material by Western blot is only possible after the death of the patient. Clinical diagnosis is a laborious process consisting of various methods and is of subordinate importance for the safety of blood donations. A summary may be found in ‘Appendix (C) Diagnosing CJD’ (pp 29).

The development of screening tests is one of the key endeavours for the safety of blood donations, especially if the testing of blood donors with a sensitive test could ensure the direct detection of persons infected with vCJD who are still in the incubation period. Such a test would be superior to indirect measures such as the prophylactic exclusion of groups of donors who had been exposed to a higher theoretical risk of vCJD.

The approaches currently being pursued for screening tests are based either on direct detection of the pathological prion protein (PrPSc), which is generally believed to correlate with TSE infectivity, in blood or other easily accessible body fluids or on the detection of other markers associated with the infection (surrogate markers).

At present, a range of tests are under development [98], but data on sensitivity and specificity are as yet largely absent. One of the problems in the detection of the PrPSc protein in body fluids is the extremely low concentration at which it naturally occurs in the periphery, if at all. The data available so far allow us to draw the analogy conclusion that for the task of detecting vCJD in human blood the sensitivity limits of currently known test systems will quickly be reached. Estimates expect considerably less than 1 pg/ml PrPSc in the blood. The most sensitive antigen tests (e.g. for the detection of HBsAg of HBV or p24 of HIV, two proteins with a molecular size similar to that of PrPSc), after many years of development and improvement, are capable of detecting antigen only at levels of 10 pg and above per milliliter plasma or serum. In addition, physiological prion protein is present in approximately 10,000-fold excess, which makes the sensitive and specific detection of PrPSc considerably more difficult. Highly specific so-called ‘conformational’ antibodies (for the recognition of PrPSc characteristic folding epitopes or conformation epitopes) therefore seem indispensable for a sensitive detection of this protein. The possible use of such an immunoassay (CDC: ‘conformation dependent immunoassay’) for clinical diagnostics of human TSE infections is currently under discussion [99].

Current research projects designed to establish a PrPSc screening test are pursuing different approaches to surmounting these limitations, e.g. attempts to increase the tests sensitivity by means of spectroscopic techniques. Other approaches use enrichment steps to increase the PrPSc concentration in the sample to be tested by selective precipitation of PrPSc through its binding to ‘ligand’ molecules or cyclical amplification of the pathogen prion protein. The artificial in vitro replication of PrPSc by means of the PMCA (protein misfolding cyclic amplification) method [100] has especially raised high expectations. However, despite demonstration of the possibility to replicate infectious PrPSc in one species by a factor of 103 [98, 101], this has not yet led to the development of appropriate test systems.

The obvious difficulties with the sensitive detection of the PrPSc prion protein, the only known specific marker of vCJD infection, have led to the exploration of alternative test concepts. A possible choice would be a screening method that allows us to use one or more markers which, alone or in combination, would permit a reliable detection of vCJD (surrogate markers). Such a screening procedure could be carried out both at the RNA level (differential display) and at the protein level (proteome analysis).

Previous analyses of the modified regulation of genes in TSE infections (differential gene expression) have shown that a number of genes are over- or under-expressed in the course of the disease. In the past few years, several working groups have examined to what extent the differential expression of genes in the course of the disease can contribute to a better understanding of the infection. In addition to a number of genes already identified which are up- or
down-regulated during a prion disease, much attention has been paid to the publication of a peripheral marker detectable in blood cells (erythroid differentiation factor; EDF) [102]. Follow-up tests, however, showed that this marker is subject to major fluctuations in healthy individuals [103]. Based on the intensive work done in this field, several surrogate markers have been published to be candidates for screening markers while proof for this remains to be provided.

Extensive examinations on well-defined populations and acceptable test features (sensitivity, specificity, high throughput) are indispensable preconditions for introducing a screening test, especially if it is to be used in blood donation screening. These conditions have so far not been met by any of the test procedures discussed in the literature. The question arises as to what extent the criteria should be established for validation. Evaluating new tests in healthy populations, e.g., blood donors, raises a number of unresolved ethical questions, such as how reactive test results are to be handled which, at least when obtained with the first available test, cannot be confirmed or clarified by another method.

### Exclusion of Certain Categories of Persons from Donating Blood

As explained in ‘Diagnosing vCJD: Screening Tests’ (see above), it is not foreseeable whether and when a routine test suitable for blood donor screening will be available that could reliably detect the agent in humans in the preclinical phase in which the pathogen concentration is probably still particularly low. The effectivity of manufacturing steps suitable to reduce potential infectivity in blood components for transfusion (e.g., prion-adsorbing blood filters) cannot currently be assessed either.

Therefore, donor selection criteria based on the history of the donor must in principle still be used for risk prevention. Such criteria have been an important part of precautionary measures for many years. An overview can be found in ‘Appendix (E) Exclusion of Transfusion Recipients: Estimating the Consequences’ (pp 34). Development and justification of the donor exclusion criteria established some years ago were described in the previous report of the working group in 2001. On May 7, 2005 the Paul-Ehrlich-Institut ordered that individuals operated on or transfused in the UK must to be excluded from donating blood. In addition, this announcement required that reference to the transmission cases observed should be made in the package leaflet and the Summary of Product Characteristics (SPC). The regulations applicable in Germany have been adopted in the hemotherapy guidelines of the Bundesärztekammer and Paul-Ehrlich-Institut [75].

Other important questions discussed in the past few years relate to whether the potential transmission by transfusion could lead to a perpetuation of vCJD among humans, even though transmission through the food chain has been stopped, and whether an exclusion of transfusion recipients could essentially influence the course of the vCJD epidemic. In several European countries, including the UK, The Netherlands, Switzerland and France – there albeit as early as 1998 under the impression of virus transmissions –, the exclusion of transfusion recipients from donating blood has meanwhile been laid down with the aim of preventing the further spread of the vCJD agent by this route. In its announcement of April 29, 2004, the Paul-Ehrlich-Institut began a graduated plan.

In order to have a scientific database for the decision, a model calculation was prepared as suggested by the working group shown in ‘Appendix (D) Model Calculation Addressing an Exclusion of Donors with a History of Transfusion’ (pp 32). The calculations are based on pessimistic assumptions. Concerning the number of individuals in Germany expected to be infected with the vCJD pathogen and potentially able to transfer this pathogen, the assumed number of 2,000 within 10 years even exceeds the estimate of the 2001 report, which today seems very pessimistic. The same is true concerning the assumed duration of the incubation period and the assumption that any contaminated blood transfusion would transmit vCJD with 100% effectivity. For a correct consideration of demographic structures, authentic data were collected of donor and recipient populations. Even though it must be anticipated that there will be vCJD transmissions by transfusion, it must also be expected that the overwhelming majority will be caused by donors infected by consumption of BSE-contaminated food. The results show that excluding transfusion recipients will not essentially change the epidemiological outcome, and even the effect of prevention in isolated cases would be minimal at best.

However, the planned exclusion of transfusion recipients would have entailed serious problems and disadvantages. The French example shows that such exclusion is possible in principle but involves major problems and efforts and can therefore be implemented only over a longer period of time. To secure the provision of blood supplies, new donors would have to be recruited to a considerable extent. In this context, it must be considered that, according to the data on epidemiology collected by the Robert Koch-Institut pursuant to Section 22 TFG, the prevalence of virus infections in new blood donors is higher than in long-term donors. Another problem is that exclusion of transfusion recipients could be perceived as a signal that, despite all efforts, blood supplies are not sufficiently safe.

In conclusion, the following can be stated: the intended measure, i.e. exclusion of transfusion recipients for the prevention of a further spread of vCJD by blood transfusions would bring about only a marginal effect, taking into account the epidemiological situation and demographic structures in Germany. The measure would, on the other hand, involve considerable disadvantages. On the basis of the present assessment of the situation, one would refrain from the measure announced on April 29, 2004.
Possible Measures for Safeguarding the Supply of Blood and Blood Products

Impact on the Amount of Blood Donations of a Deferral of Transfusion Recipients

New reasons for the exclusion of individuals willing to donate blood with effects on the safeguarding of the blood supply would require a risk/benefit assessment (safety vs. blood supply). Blood donation services have some experience in the effects of donor deferrals on the donation availability. Some new donor deferral criteria had been introduced, for instance, with the update of the guidelines by the Bundesärztekammer and the Paul-Ehrlich-Institut in 2000. In this context, the increase in the Hb limit for men to 135 g/l has had a serious effect on the donor population. The deferral rate initially rose by approximately 2.5% and, after the male donors with an Hb between 125 and 135 g/l were removed from the donor population, decreased again to its initial level (source: German Red Cross (GRC) Blood Donation Center West, figures from North-Rhine Westphalia).

The result is that the number of actual donations has fallen. This effect is enhanced by deferral rates which tend to be rising (2002: 322,312 donors corresponding to 8.25% of the population prepared to donate, vs. 2003: 345,906 donors, corresponding to 8.87% of the donors willing to donate) – source: Statistischer Jahresbericht (Statistical Annual Report 2003 of the German Red Cross blood donation centers). Since winter 2000/2001 – despite intensive encouragement to donate blood – the demand for blood components, especially RBCC has not always been met so that planned operations, for example, have to be postponed.

On the other hand, more strict donor exclusion criteria for the sake of improved safety have resulted from new scientific findings, which have led to a loss in donors. The implementation of Recommendation 31 of the Arbeitskreis Blut for anti-HBc testing [104], for example, has led to the permanent exclusion of approximately 0.6–1% of blood donors, depending on the test system, unless the donor population had already been routinely tested for anti-HBc, and, in the case of a positive result, excluded from future blood donations.

Each new reason for exclusion frequently results in a lack of understanding on the part of those concerned and requires great efforts in informing these individuals. As the experience with the deferral due to a cumulative stay in the UK of more than 6 months between 1980 and 1996 has shown, considerable uncertainties remain for the donor due to the lack of possibility to explore his individual risk of infection or to obtain a confirmatory/exclusion test despite numerous dialogues.

Besides, it can be expected that an undetermined number of donors with a transfusion history not known or not remembered may continue to donate blood so that only a fraction of potentially unsafe donations can be avoided by deferring donors. On the other hand, no donor screening test that is reliable in practice will be available in the foreseeable future for the recognition of potential transmitters of vCJD so that a deferral of transfusion recipients is the only way of eventually reducing the risk of a further transmission of vCJD by transfusion.

Before introducing any further donor deferrals, however, their effects should be quantified, and the prospects of success for any correcting measures must be accurately assessed. Also the time factor must be taken into account: while special promotion campaigns (cf. bone marrow/stem cell donors for children with leukemia) are able to motivate many people to donate on a short-term basis, it is the continued reliability of donor preparedness in connection with the constantly required readiness to act that is important in blood donor promotion campaigns.

The exclusion of transfusion recipients from donating blood, however, cannot prevent cases such as the three probable transmissions in the UK (cf. ‘The Occurrence of vCJD’, pp 7; ‘Risk of Transmission of vCJD through Blood (Secondary Infections)’, pp 13) since the donors whose blood components probably transmitted vCJD were not transfusion recipients themselves.

The following possible approaches could secure continued donor preparedness and the supply of blood components:

– most economical use of blood and blood products,
– sustained recruitment measures,
– increasing the social prestige of the blood donors,
– further harmonization of the interpretation of exclusion criteria,
– reduction in the number of products required for quality control.
Most Economical Use of Blood and Blood Products

All measures that result in an optimal use of blood and blood products will not only minimize the possible transmission risk but will also contribute, thanks to saving, to safeguarding the supply of blood and blood products. The activities in Germany and the European Union have been described in detail in ‘Measures for the Optimal Use of Blood Products’ (pp 19). An important instrument available in the Federal Republic of Germany is the consistent adherence to the guidelines for therapy with blood components and plasma derivatives [96] as state of the art. In addition, the analysis of the French experience with the compensation of donor losses due to deferrals of donors with a transfusion history can help to limit the effects of the measures by, among other things, an even more specific use of heterotherapeutics.

Sustained Recruitment Measures

The German Red Cross blood donation services are currently spending approximately 20 million EUR a year on maintaining their existing donor base. Additional recruitment campaigns (approximately 3 million EUR annually) have so far been aimed at the approximately 1–2% increase in donor figures that is required to compensate for the annual rise in demand resulting from the increasing average age of the population. They have been able to fulfill this goal with a relatively low budget since advertising space in the various media has generously been made available to the GRC free of charge.

In the case of donor exclusion due to transfusion history, an appropriate blood donor campaign will be required in order to bring about an immediate and sustained increase in willingness to donate, e.g. in the event of donors who, since 1980, have had a transfusion be excluded. Just under 18,000 additional volunteer donors per month will be needed in the first half-year, and more than 11,800 additional volunteer donors per month in the second. Altogether, approximately 4 first-time volunteers will have to be recruited for each deferred donor (cf. ‘Appendix (E) Exclusion of Transfusion Recipients: Estimating the Consequences’; pp 34). Governmental measures can be very effective in increasing the acceptance of blood and plasma donation in the population. This additional recruitment campaign can no longer be carried out by recruitment measures used thus far by the blood donation services, requiring more financial means since the blood donations will have to be determined for fixed time intervals. The existing blood donor recruitment campaign by the GRC donation services, for example, is based on pro bono decisions if the media have free capacities and leaves little room for time limits.

The costs of an appropriate recruitment campaign can only be estimated on the basis of comparable campaigns in the past.

– The ‘Aktion Mensch’ organization spent around 10 million EUR in the second half of 2000 on its campaign to introduce its new name. The effect of the campaign had, however, already evaporated by January 2001.

– At the start of its campaigns against AIDS, the Federal Center for Health Education (Bundeszentrale für gesundheitliche Aufklärung; BZgA) worked with an annual budget on the order of 25 million EUR.

Previous recruitment campaigns by the GRC blood donation services in the amount of approximately 3 million EUR annually as well as the ‘Kleine Aktion’ (Small Campaign) by the BZgA have been and still are insufficient to compensate for an acute shortage. If, therefore, an additional reason for donor exclusion is introduced and this has serious consequences, a considerable increase in recruitment expenditure will be required in order to replace the lost donations and to safeguard the supply of blood preparations.

Increasing the Social Prestige of Blood Donors

It might be helpful to provide donors with a non-material reward in the form of increasing the social prestige attached to the act of giving blood. A professional study should examine the possibilities and develop suggestions for raising the esteem in which blood donors are held. In this context, potential is seen in the non-material recognition of the blood donors’ dedication by regional and national governments (press, radio, TV, honors celebrations with a PR effect), in parallel with the awards for donation services.

Further Approximation of the Interpretation of Exclusion Criteria

According to statistics from the GRC blood donation services, the deferral rate among all volunteer donors in 2003 was 8.87% (table 8).

Compared with the GRC statistics for 1999, the strong fluctuation in the deferral rate between the various blood donation services has decreased. It ranged from 5–14% and is now 7–12%, with the exception of Berlin (highest value). No correlation can be detected between the deferral rate and the degree of conurbation. These
differences can certainly not solely be explained by differences in the donor population of the GRC blood donation services. Another improvement in harmonizing the interpretation of deferral reasons could be the introduction of uniform interpretation aids throughout Germany.

Reduction of the Amount of Components Needed for Quality Control

Since the quality of blood components (RBCC, platelet concentrates, FFP) has been proven, a reduction seems possible in the number of required quality controls involving destruction of the product.
Appendices

Appendix (A) Effect of Pool Size on the Potential Risk of vCJD Transmission

The risk of a vCJD transmission by blood plasma or blood plasma products depends on various factors:
- the probability that at least one donor in the plasma pool is infected with vCJD (risk of contamination),
- the probability that the recipient of a product from such a pool is exposed to the infectious agent (risk of exposure),
- the probability that an infection with vCJD arises as a result of the exposure (risk of infection).

While the risk of infection cannot be derived from statistical analysis, it is relatively easy to determine both the effect of donor prevalence on the contamination risk of a plasma pool and the risk of exposure for a recipient of products from a given contaminated plasma pool. For a single dose the risk of coming into contact with the agent is equal to the product of the contamination risk and the exposure risk. If the quantity of agent in a contaminated pool exceeds the number of doses manufactured from it, the risk of exposure is equal to 1. According to the current state of knowledge, however, this precondition is not met in the case of (v)CJD agents, as explained below.

Effect of Donor Prevalence on the Risk of Contamination of a Plasma Pool

For a given prevalence p for the occurrence of vCJD in the blood donor population and a plasma pool of size n, the probability that at least one patient infected with vCJD is included in this pool and that the pool is thus contaminated is

\[
r = 1 - (1 - p)^n. \tag{1}
\]

This probability represents the risk of a patient receiving a product from a contaminated pool if a single dose is given. For patients receiving products from m different pools (for simplicity’s sake assumed to be the same size n), the probability that at least one of these pools is contaminated can be calculated according

\[
r_m = 1 - (1 - p)^{nm}. \tag{2}
\]

Obviously the risk of contamination rises as the pool size increases.

Equations (1) and (2) can be used to calculate the maximum pool size \( n_{\text{max}} \) at which the risk of contamination, i.e. the risk of a recipient of blood products receiving products from a contaminated pool, will not exceed a prespecified threshold \( r_{\text{accept}} \):

\[
n_{\text{max}} = \log(1 - r_{\text{accept}})/\log(1 - p),
\]

with a single application or

\[
n_{\text{max}} = \log(1 - r_{\text{accept}})/(m\log(1 - p)) \tag{4}
\]

with m applications.

These relationships are illustrated below. It has to be noted that at present no valid data are available on the prevalence of vCJD in Germany. Thus, with regard to prevalence the following data are hypothetical in nature and serve only for illustration.

Table 9 presents the risk of receiving contaminated blood products as function of pool size and number of different pools. According to this, even with small pools patients who regularly receive blood products are exposed to a non-negligible risk of receiving products from at least one contaminated pool.

If the risk of coming into contact with contaminated blood products should fall below a prespecified limit, the maximum allowable pool size (depending on the number of applications from different pools) is shown in table 10.

Figure 2 shows a graphical representation of the relationship between the maximum pool size and the maximum acceptable risk of contamination:

In fact, a reduction in the pool size significantly reduces a possible (already relatively low) risk of contamination with vCJD for the individual patient. However, if a patient is exposed to a larger number of pools, the effect of pool size is only modest.

Risk of Exposure to a Recipient from a Contaminated Plasma Pool

The following assumptions are made:
- An infectious unit behaves like a particle, i.e. concentration has no effect on infectivity. As mentioned above, this condition may not be true for TSE agents insofar as aggregates may dissolve at higher dilutions and thus no longer be infectious. The assumption made here therefore describes the less favorable case.
- Recipients of the products manufactured from a pool receive equal quantities, with one unit corresponding to the volume of one donation (250 ml).
The concentration of infectious units in the plasma is assumed...

**Appendix (B) Nanofiltration**

Preliminary studies on the efficiency of nanofiltration in removing TSE agents have been carried out by a number of filter manufacturers and plasma fractionators. They have mainly been presented to conferences and are as yet unpublished. It is difficult to evaluate and compare these studies since the following test conditions varied:

- filters from different manufacturers with different pore sizes,
- filtration of different products (albumin, coagulation factors, immunoglobulin),
- spiking with different strains of TSE,
- different preparation and processing of the TSE material (brain homogenate, fibrils, with and without addition of detergent),
- different detection systems (bioassay in mouse or hamster, PrPSc tests).

In its main conclusion, the results of the study by Tateishi et al., [80] (table 13) correspond to those of other trials based on different approaches: PrPSc could be retained by a 35-nm filter when the...
The studies available to date are not sufficient to allow a general
effectivity of pathogen removal strongly depends on the state
in an experiment)


determination: 10 animals used per dilution


filtration was carried out without the addition of detergent. The
addition of detergent obviously caused the aggregates to dissolve,
thus allowing PrP°Sc to pass through the filter so that considerable
amounts of PrP°Sc could be detected in the filtrate after filtration
through a 40-nm or 35-nm filter [107].

Other experiments showed that most of the PrP°Sc was retained by
a 75-nm membrane, even though detergent had also been added. It
was nonetheless determined, in accordance with the aforementioned
study, that PrP°Sc that had passed through the 75-nm membrane
was retained only in negligible quantities by the 35-nm membrane
and that reliable effectiveness (> 2 log10 in an experiment)
could only be achieved using filters with a lower pore size (15 nm).
However, until now unpublished experiments were reported at
conferences according to which, after treatment with ultrasound
filtration or whether the same results may be expected for spiking
with infectious vCJD material.

The various studies allow the following conclusions to be drawn:

- Effectivity of pathogen removal strongly depends on the state
  of aggregation of the material used in the experiments. The
  state of aggregation in which the potential pathogen is present
  in human blood or plasma is currently unknown. It is also un-
  known to what extent aggregation of the pathogen takes place
during the different steps of plasma fractionation. The addition
of detergent appears to dissolve aggregates PrP°Sc preparations,
PrP°Sc in absence of detergent can more easily be removed by
filtration.

- PrP°Sc preparations manufactured solely by homogenization of
  infected brain tissue or by preparation of fibrils can be partly
  removed by filtration even when a membrane with a larger aver-
  age pore size (e.g. 75 nm) is used. It is not clear, at present,
  whether this represents an overvaluation of the effectiveness of
  filtration or whether the same results may be expected for spik-
  ing with infectious vCJD material.

- The studies available to date are not sufficient to allow a general
  assessment to be made about the effectiveness and reliability of
  nanofiltration in the elimination of PrP°Sc. The properties of
  the PrP°Sc preparation are particularly important in an examination
  of the effectiveness of filtration. Furthermore, additional studies
  are required to test whether the provisional findings summarized
  above can be applied to the vCJD agent.

---

**Table 11.** Risk of exposure following a single application as function of pool size

<table>
<thead>
<tr>
<th>Pool size</th>
<th>Risk of contamination, %</th>
<th>Expected number of IU i. v. in a contaminated pool (rounded up)</th>
<th>Probability of receiving at least 1 IU i. v. from the contaminated pool, %</th>
<th>Risk of exposure, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>10,000</td>
<td>7.9956</td>
<td>261</td>
<td>2.576</td>
<td>0.206</td>
</tr>
<tr>
<td>1,000</td>
<td>0.8299</td>
<td>252</td>
<td>22.276</td>
<td>0.185</td>
</tr>
<tr>
<td>100</td>
<td>0.0833</td>
<td>251</td>
<td>91.873</td>
<td>0.077</td>
</tr>
<tr>
<td>10</td>
<td>0.0083</td>
<td>251</td>
<td>100</td>
<td>0.008</td>
</tr>
</tbody>
</table>

**Table 12.** Risk of exposure following 10 applications from the same pool as function of pool size

<table>
<thead>
<tr>
<th>Pool size</th>
<th>Risk of contamination, %</th>
<th>Expected number of IU i. v. in a contaminated pool (rounded up)</th>
<th>Probability of receiving at least 1 IU i. v. from the contaminated pool, %</th>
<th>Risk of exposure, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>10,000</td>
<td>7.9956</td>
<td>261</td>
<td>22.972</td>
<td>1.837</td>
</tr>
<tr>
<td>1,000</td>
<td>0.8299</td>
<td>252</td>
<td>91.954</td>
<td>0.763</td>
</tr>
<tr>
<td>100</td>
<td>0.0833</td>
<td>251</td>
<td>100</td>
<td>0.083</td>
</tr>
<tr>
<td>10</td>
<td>0.0083</td>
<td>251</td>
<td>100</td>
<td>0.008</td>
</tr>
</tbody>
</table>

**Table 13.** Results of the study by Tateishi et al., 2001 [80]*

<table>
<thead>
<tr>
<th>Planova type (pore size)</th>
<th>Scrapie titer challenge (log10 ID50)</th>
<th>Scrapie titer filtrate (log10 ID50)</th>
<th>Reduction factor (log10 Rf)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2% albumin + 1.5% brain homogenate</td>
<td>5.71</td>
<td>&lt; 2.26</td>
<td>&gt; 5.87</td>
</tr>
<tr>
<td>2% albumin + 1.5% brain homogenate + 0.5% sarcosyl</td>
<td>5.71</td>
<td>&lt; 2.26</td>
<td>&gt; 5.87</td>
</tr>
</tbody>
</table>

*Test conditions: Mouse-adapted ME7 scrapie strain, titration in C57Bl/6 mice, test solution (1) 2% albumin solution, with addition of 1.5% brain homogenate in PBS, (2) as for (1) plus addition of 0.5% sarcosyl, filters: Planova 35N (~35 nm pore size), Planova 15N (~15 nm pore size), Planova 10N (~10 nm pore size), titer determination: 10 animals used per dilution (20 µl, i.c.), observation maximally 20 months for clinical and histological changes.
Possible CJD is diagnosed if the clinical criteria are fulfilled, if, however, there is neither a positive EEG or a positive liquor.

Probable CJD
Progressive dementia and at least two out of the following clinical features:
- Myoclonus
- Visual or cerebellar disturbance
- Pyramidal/extrapyramidal dysfunction
- Akinetic mutism
Periodic sharp waves in the EEG or
A positive protein 14-3-3 assay in the liquor and clinical duration to death < 2 years

Possible CJD is diagnosed if the clinical criteria are fulfilled, if, however there is neither a positive EEG or a positive liquor.

**Appendix (C) Diagnosing CJD**

**Clinical Parameters**

According to the currently applicable diagnostic criteria, a certain CJD diagnosis requires a neuropathologic examination of the brain tissue. However, advances in new techniques and methods mean that the combination of clinical symptoms and signs together with a number of additional tests allows a reliable clinical diagnosis of CJD as well. The combination of clinical symptoms and signs together with a number of additional tests allows a reliable distinction between CJD and other neurodegenerative diseases (table 14). Increasing experience with the symptoms of vCJD also helped establishing clinical criteria for this type of disorder (table 15).

A number of additional tests is used to support the clinical diagnosis. The oldest method in this respect is the electroencephalogram (EEG). Newer processes such as magnetic resonance imaging (MRI) and analysis of the cerebrospinal fluid are, however, far superior to the EEG. In the differentiation of vCJD from the sporadic form (sCJD), MRI plays an important role.

**EEG**

Characteristic EEG changes in sCJD were described for the first time in the 1950s [112]. Since then periodic bi- and triphasic complexes (periodic sharp and slow wave complexes; PSWCs) have been considered an electroencephalographic pattern that may assist diagnosis. In the early stages of the disease the detection of typical EEG changes does not correlate with the severity of the clinical picture, but early occurrence is associated with a shorter survival time. PSWCs are seen in the course of the disease in approximately 60–70% of cases of sCJD [113, 114]. By contrast, no such changes have so far been observed in vCJD (fig. 3).

**MRI**

MRI is one of the methods for the diagnostic clarification of rapidly progressive dementia. In addition to excluding other diagnoses, this method can provide findings to support the clinical suspicion of CJD. In sCJD, hyperintensities are observed in the caudate nucleus and putamen in approximately two thirds of cases. In this respect, so-called diffusion-weighted imaging has proved to be superior to FLAIR, T2 and proton weighting [115, 116]. The special value of MRI lies in the possibility of distinguishing vCJD: with vCJD the strongest signals are seen in the posterior thalamus (the ‘pulvinar sign’). Since this signal pattern has been observed in 78% of vCJD cases, MRI has become one of the diagnostic criteria for vCJD [111] (fig. 4).

**Cerebrospinal Fluid**

The standard cerebrospinal fluid (CSF) parameters (cell number, barrier function, inflammatory reaction) are generally normal in patients with CJD [117]. The rapid neuronal destruction or astrocytic activation results in brain proteins crossing into the CSF. Proteins such as neuron-specific enolase (NSE), S100b protein, tau, brain-specific creatine kinase and G0 protein have been measured in abnormally high concentrations in the CSF in cases of CJD [118, 119]. Increased concentrations of these proteins represent an indicator for a rapidly destructive neuronal process and therefore assist in the differential diagnosis of sCJD and other neurodegenerative diseases. At present, the most important test is the detection of 14-3-3 proteins in the CSF. In the differential diagnosis of dementia, this test gives a sensitivity of 94% with a specificity of 93%.

---

**Table 14. Diagnostic criteria for sporadic CJD [108, 109]**

<table>
<thead>
<tr>
<th>Definite CJD</th>
<th>Probable CJD</th>
</tr>
</thead>
<tbody>
<tr>
<td>By neuropathological examination including identification of PrPSc by immunohistochemical imaging with specific antibodies or by detection of PrPSc in Western blot.</td>
<td>Progressive dementia and at least two out of the following clinical features:</td>
</tr>
<tr>
<td>- Myoclonus</td>
<td>i) Myoclonus</td>
</tr>
<tr>
<td>- Visual or cerebellar disturbance</td>
<td>ii) Visual or cerebellar disturbance</td>
</tr>
<tr>
<td>- Pyramidal/extrapyramidal dysfunction</td>
<td>iii) Pyramidal/extrapyramidal dysfunction</td>
</tr>
<tr>
<td>- Akinetic mutism</td>
<td>iv) Akinetic mutism</td>
</tr>
<tr>
<td>Periodic sharp waves in the EEG or</td>
<td>and</td>
</tr>
<tr>
<td>A positive protein 14-3-3 assay in the liquor and clinical duration to death &lt; 2 years</td>
<td>Possible CJD</td>
</tr>
<tr>
<td>‘Possible CJD’</td>
<td>Possible CJD is diagnosed if the clinical criteria are fulfilled, if, however there is neither a positive EEG or a positive liquor.</td>
</tr>
</tbody>
</table>

**Table 15. Diagnostic criteria for vCJD* [110, 111]**

| I | a) Progressive neuropsychiatric disorder |
| b) Duration of illness > 6 months |
| c) Routine investigations do not suggest an alternative diagnosis |
| d) No history of potential iatrogenic exposure |
| e) No evidence of a familial form of TSE |
| II | a) Early psychiatric symptoms* |
| b) Persistent painful sensory symptoms |
| c) Ataxia |
| d) Myoklonus or chorea or dystonia |
| e) Dementia |
| III | a) EEG does not show the typical appearance of sporadic CJD (or no EEG performed) |
| b) MRI brain scan shows bilateral symmetrical pulvinar high signal |
| IV | a) Positive tonsil biopsy** |

*Definite: Ia and neuropathological confirmation of vCJD. Probable: I and 4/5 of II and IIIa and IIIb or I and IVa. Possible: I and 4/5 of II and IIIa.

**Tonsil biopsy is not recommended routinely, nor in cases with EEG appearances typical of sporadic CJD, but may be useful in suspect cases in which the clinical features are compatible with vCJD and where MRI does not show a bilateral pulvinar high signal.**
[109, 119, 120] (table 16). In contrast to sCJD, in case of vCJD increased 14-3-3 concentrations in the CSF were found in only 45% of patients [121].

The detection of the CJD-typical proteinase K-resistant form of the prion protein in the CSF and potentially in blood would lead to an in vivo diagnostic test; this is, however, still under development. The tests available to date do not allow the preclinical diagnosis of CJD. They often show positive only in the advanced stages of the disease, for example EEG. Examination of the CSF with the detection of neural and astrocytic proteins as an expression of rapidly
progressive neural destruction is currently the most sensitive method in the clinical and differential diagnosis of sCJD. Even in the early stages, 14-3-3 proteins can frequently be detected, often at a time when the first signs of the disease have already appeared but before the typical full clinical picture has emerged.

**Neuropathology**

Neuropathological diagnosis is based on histological identification of spongiform changes of neuropil, loss of neuronal cells, and gliosis. The degree of morphological changes varies depending on the case and the region of the brain concerned. In isolated cases, Kuru plaques are already detectable by conventional light microscopy. The neuropathologic diagnostic standard includes the identification of pathological prion protein plaque in the brain [123]. This identification is possible using immunohistochemistry, paraffin-embedded tissue (PET) blot and Western blot (see below). Detection of PrPSc by immunohistochemistry allows a differential diagnosis of CJD in different subgroups that corresponds very well to the biochemical detection of the different PrP types in the Western blot and in genetics [124]. Definite differentiation of vCJD from other subgroups of sCJD and iatrogenically transmitted CJD (iCJD) can be performed histologically and by immunohistochemistry. PET blot examination can routinely be carried out in all formalin-fixed brain samples. PET is transferred to a nitrocellulose membrane, and PrPSc is sensitively detected after proteinase K digestion by means of specific antibodies [125].

Diagnostic Western blot from deep-frozen brain tissue or lymphatic tissue is an integral part of routine CJD diagnostics. Several types of PrPSc can be classified by their biochemical properties which are associated with different clinical outcomes [122]. Variant CJD can be clearly distinguished from other types on the basis of its special glycosylation pattern. Particular familial forms of spongiform encephalopathy (familial CJD (fCJD), GSS, fatal familial insomnia (FFI)) show further characteristics which, in combination with clinical pathology and histological findings, are of major diagnostic significance.

For biochemical examination by Western blot, very small tissue samples are homogenized and exposed to enzymatic digestion in order to show PrPSc by its particular resistance to proteinase K. In the subsequent gel electrophoresis, the three different glycosylation forms are separated. Differences are shown in the pattern of different PrPSc strains which can be detected after transfer to PVDF membranes (specific antibodies, indirect enzymatic detection method). At least three types, PrPSc I, IIa and IIb, can be detected using the current international classification. These types are classified in combination with the polymorphism at codon 129 and can be associated with particular neuropathological changes and clinical outcomes.

The appearance of vCJD in the Western blot is characteristic and clearly distinct from all described forms of sCJD. Notari et al. [126] were able to show that by limiting proteinase K digestion under acidic pH values, fine differences in the resistance of PrPSc became visible, going beyond the previous classification scheme. This may indirectly enable a more accurate allocation to the previously described six clinical and pathological CJD types by protein conformation and may even make further subtypes recognizable. This is relevant in epidemiologic examinations and strain typing (assignment of the disease to different prion strains) in human prion diseases.

**Genetics**

The etiology of prion disease in humans is unknown in a large portion of cases (approximately 90%); these are called idiopathic or, on the basis of their epidemiological occurrence, sCJD. For a small portion of the cases (<1%), an infection can be assumed as the cause; these are the iCJD and the cases of vCJD. In approximately 10% of the cases, a mutation of the prion protein gene (PRNP) is found as the cause. These congenital diseases are called ICJD, GSS syndrome, or FFI, depending on their clinical or pathological manifestation. 32 different mutations have so far been described that have been associated with fCJD, GSS, or FFI, and are autosomal dominantly inherited. The probability of developing a prion disease (penetrance) is nearly 100% for the carrier. Clinical and pathological manifestation can vary considerably from patient to patient depending on the respective mutation; it can generally be stated that clinically atypical cases of prion disease are frequently caused by a mutation of PRNP.

Apart from the detection of pathologic mutations in genetic cases of prion diseases, polymorphisms in PRNP are detected by genetic analysis. Most of these polymorphisms are very rare and have no recognizable influence on the disease. The polymorphism at codon 129 of PRNP (methionine or valine) may solely modify both the probability to develop the disease and the clinical course and pathology of the disorder.

Analysis of PRNP is imperative in all suspected cases of prion disease for several reasons. It is necessary in order to identify cases of congenital prion disease. Since many affected families are not aware of the fact of a predisposition for dementia, an interview on family history alone may not be sufficient to identify congenital cases. On the other hand, of course, we can only talk about a sporad-

---

**Table 16. Tests in CJD**

<table>
<thead>
<tr>
<th>Test</th>
<th>Sporadic CJD, %</th>
<th>vCJD, %</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>sensitivity</td>
<td>specificity*</td>
</tr>
<tr>
<td>CSF</td>
<td>94</td>
<td>93</td>
</tr>
<tr>
<td>PrPSc**</td>
<td>20</td>
<td>100</td>
</tr>
<tr>
<td>MRT</td>
<td>63</td>
<td>92</td>
</tr>
<tr>
<td>EEG</td>
<td>66</td>
<td>74</td>
</tr>
</tbody>
</table>

n. e. = Not examined.

* In the differential diagnosis of relevant diseases.

** Fluorescence correlation spectroscopy, SIFT [122].

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Appendices
ic or infection-based prion disease after a mutation in PRNP has been ruled out. Atypical forms of prion diseases are frequently caused by mutations in PRNP and can therefore only be identified by a comprehensive analysis of clinically improbable cases. The discovery of new mutations could lead to new types of prion disease with clinical outcomes and pathologies clearly distinct from the known spectrum. The best example of this is represented by FFI which has only been recognized as congenital prion disease since 1992 and is now of major significance within the group of congenital prion diseases. With the known mutations, too, each newly discovered case will lead to a gain in information on the connection between a particular mutation and a particular clinical and pathological manifestation.

PRNP analysis also makes possible differentiation from other demyelinating diseases which, to a certain extent, occur as familial disorders, e.g. all hereditary forms of Alzheimer’s disease. In each sporadic case of a prion disease, polymorphism at codon 129 of PRNP must be analyzed since the clinical and pathological manifestation of sCJD is essentially influenced by this polymorphism. Finally, diagnosis of vCJD also requires a PRNP analysis since a pathogenic mutation that could lead to a pathological manifestation similar to that of the vCJD must be ruled out. In addition, polymorphism at codon 129 must be determined since this is an important parameter in assessing and classifying previous cases of vCJD.

Appendix (D) Model Calculation Addressing an Exclusion of Donors with a History of Transfusion

The epidemiological model describes the spreading of an infection, in this case vCJD, due to blood donations based on the demographic situation in Germany. It assumes that 2,000 individuals were infected by contaminated food during a limited period of 10 years. The total population comprises 80 million people. The parameters for the model were estimated on the basis of four data sets:

- donations from 262,071 donors at the blood donation services of DRK (DRK-Blutspendedienst) West, Hagen, Germany,
- 617 controls of a case control study on CJD at Göttingen University, Germany,
- age distribution of 1,343 transfusion recipients at the University Hospital of Essen, Germany, and
- a longitudinal study from Newcastle on the survival of 2,888 patients after a blood transfusion in June 1994.

The age-structured model uses 2-month intervals and takes into account the following conditions:

- The mandatory age limit for blood donors is between 18 and 68 years. Each blood donor undergoes an active phase of donor activity the duration of which depends on age.
- The risk of receiving a blood transfusion strongly depends on age and has its peak at approximately 70 years.
- Survival after a blood transfusion also strongly depends on age. The increased mortality rate of transfusion recipients reduces the risk of spreading by blood donations.

- The model takes into account the current mortality rates in the Federal Republic of Germany.
- A mean incubation period of 16 years with a standard deviation of 4 years was assumed for the infection.
- The model permits exclusion of donors with a history of blood transfusions. It is assumed that 95% of the donors with a history of blood transfusions can be excluded.

Figure 5 shows the absolute infection prevalence as a function of time predicted by the model. Prevalence increases during the 10-year period of food-related infection and leads to a maximum of 1,434 infected individuals in the portion of the population without transfusion history (curve A). In the portion of the population with transfusion history, depending on whether no risk of infection is assumed (0%, curve B) or, in the most unfavorable case, an absolute infection risk is assumed (100%, curve C) by blood donations from...
infected donors, 426 or 504 infected individuals respectively are to be expected. The maximum prevalence in the German population is 1,860 or 1,921 infected individuals, corresponding to approximately 24 infected individuals per 1 million inhabitants. (The maximum value of 1,921 is slightly smaller than the total of 1,434 plus 504 since the maximum values of the individual curves are reached at different times). The majority of infections caused by transfusions cannot be prevented by the exclusion of donors with a transfusion history since they were infected by blood from food-infected donors without a transfusion history. Thus, an exclusion of transfusion recipients would bring about only a minor contribution to prevention (curves C and D can hardly be distinguished).

Given the initial rate of introduction of the infection, no further spreading occurs after that period of time, and, due to decreased life expectancies of vCJD-infected individuals, the prevalence during the subsequent 20–30 years has a tendency towards zero again. Even if it is assumed that infected blood donations will always lead to infection of the recipient (infection risk = 100%), no further spreading occurs. In addition the decrease in prevalence is only delayed due to the incubation period of the individuals infected (curve C). An exclusion of transfusion recipients, even in the latter most pessimistic scenario, can bring about only a minor contribution to prevention (curve D). This is also shown in figure 5b in which the annual incidence of deaths due to infection is shown. The maximum number of vCJD-associated deaths occurs not before 23 years after the beginning of the onset of infection due to the long incubation period.

Figure 6 compares the incidence of deaths of individuals infected by food (curve E) with those that may be caused by blood donations with maximum risk of infection (curve F). Due to the incubation period, transfusion-associated deaths occur markedly later than deaths caused by food infections. Within the displayed 50 years 172 transfusion-associated deaths have to be expected. During this period, however, a maximum 15 cases could be prevented if donors with a transfusion history were excluded, equivalent to 1 case in 3–4 years. Out of the 2,000 individuals infected by food, we expect 1,557 vCJD cases if the infection risk of infected donors is 0%, and 1,729 cases, if the infection risk is 100%. If approximately 20% of the donors were excluded, less than 1% of the cases would be prevented.

Figure 7 explains why the exclusion of donors with a transfusion history only slightly influences the incidence of deaths:

- The majority of the infected donors were infected by food and reveal no transfusion history (curve A). This group is not covered by the exclusion criterion ‘donors with a transfusion history’, and is able to continue to transmit the disease.
- Infected donors with a transfusion history can be excluded but only present a minor portion of infected donors (curve B or C).
- The portion of donors infected by transfusions is very small (difference between curve C and curve B).

The assumptions chosen here present a considerable overestimation of the real risk of infection in Germany. Since an infection introduced by food cannot be sustained in the population, there is no further long-term risk after this route of transmission has been interrupted. Because of the low prevalence of approximately 24 infected individuals per 1 million (see above), linear reduction can be performed on predicted developments if markedly lower prevalences are assumed. The actual prevalence of individuals infected by food in Germany is probably lower by at least a factor of 10. Therefore, the above mentioned figures may probably be reduced by the corresponding factor. A detailed description of the model with all parameters and figures for the data sources on which this calculation is based has been submitted for publication (Dietz K, Raddatz G, Wallis J, Müller N,
Appendix (E) Exclusion of Transfusion Recipients: Estimating the Consequences

To investigate how many people in the German blood donor population indicate or cannot rule out having had previous blood transfusions, a survey of 4,838 donors in Germany was carried out in January/February 2001.

The study was limited to questions on blood or plasma transfusions (red cells, blood plasma, blood platelets, autologous blood, and exchange of blood) in the period after 1980, since a preliminary survey had indicated that asking about the whole range of blood products would not yield usable results.

The study report (Storch and Schindel, 2001) was presented to the National Advisory Committee ‘Blood’ of the German Federal Ministry of Health on March 1, 2001: Up to 4% of repeat donors who give blood on a more or less regular basis (according to the study 2–3 times a year) would be affected by the deferral measure.

Table 17. Total amounts of blood products manufactured by GRC and StKB in the year 2003

<table>
<thead>
<tr>
<th>Product Type</th>
<th>GRC</th>
<th>StKB</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Manufactured red blood cell concentrates</td>
<td>3,119,135</td>
<td>956,839</td>
<td>4,075,974</td>
</tr>
<tr>
<td>FFP/VIP from whole blood 250 ml* units GRC</td>
<td>467,244</td>
<td></td>
<td></td>
</tr>
<tr>
<td>FFP from whole blood 250 ml* units StKB</td>
<td>492,932</td>
<td></td>
<td></td>
</tr>
<tr>
<td>FFP/VIP from apheresis 250 ml* units GRC</td>
<td>183,992</td>
<td></td>
<td></td>
</tr>
<tr>
<td>FFP from apheresis 250 ml* units StKB</td>
<td>190,276</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Apheresis platelet concentrates GRC</td>
<td>46,576</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Apheresis platelet concentrates StKB</td>
<td>160,104</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Plasma for fractionation GRC, l</td>
<td>984,780</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Plasma for fractionation StKB, l</td>
<td>179,370</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total, l</td>
<td>1,164,150</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

GRC = German Red Cross; StKB = State/Municipal Blood Donation Services; FFP = fresh frozen plasma; VIP = virus-inactivated plasma.

* To ensure comparability, the figures were converted to a ‘standard unit’ of 250 ml.

Estimating the Consequences of Excluding 4% of Donors

For an estimation of the risk to the supply, figures are available for 2003 (GRC statistics, statistics of blood banks of universities and hospitals (Arbeitgemeinschaft der Ärzte staatlicher und kommunaler Bluttransfusionsdienste e.V.; StKB). Delivered products from own manufacture are taken into account (table 17).

Effects on Whole Blood Donors/Donations

Assuming whole blood donors give blood twice a year on average, a shortage of 4% of the annually collected donations would result, if the donor referral rate was 4%, corresponding to:

- 163,039 RBCC,
- 38,407 FFP/SD-P (250 ml units) from whole blood,
- 40,048 l plasma for fractionation (Plasma for fractionation from whole blood donations: GRC 875,800 l + StKB 125,392 l = 1,001,192 l; 4% from this figure = 40,048 l).

Assuming that – based on the decreasing requirement for plasma for fractionation – the number of released whole blood donations to be compensated for is determined by the number of manufactured RBCC and FFP, 163,039 whole blood donations per year would have to be obtained from new donors. The question arises how many first-time volunteer donors must be motivated to meet this donation requirement (assumptions: the portion of repeat donors increasing their number of donations per year is negligible. Multiple donors provide one donation in the first and another one in the second half-year). The following parameters will have to be taken into account:

- Deferral rate of first-time volunteer donors who appeared: 18.6% (59,734 of 320,370 first-time volunteer donors who appeared – Statistic Annual Report 2003 of the GRC blood donation center) + 4% on the basis of transfusion history = 22.6%.
- Deferrals based on laboratory results: 1%.
- Only approximately one third of the first-time donors give blood for a second time (survey by GRC Blood Donation Center West).

In addition to the first-time donors required for compensating for the ‘normal’ donor losses, approximately 106,700 additional first-time volunteer donors would have to be motivated to donate whole blood within the first 6 months after introducing an exclusion of donors with a history of transfusion (106,700 – 25,181 (23.6%) = approximately 81,520, corresponding to 50% of the 163,039 whole blood donations to be compensated for).

Two thirds of the approximately 81,520 (suitable) new donors would not donate blood for a second time. Therefore, 54,346 suitable donations would again have to originate from first-time donors during months 6–12, corresponding to approximately 71,200 additional first-time volunteer donors, assuming the deferral rate is 23.6%.

Therefore, approximately 177,900 additional first-time volunteer donors would be required in 1 year to sustain the supply (which is currently not sufficient under all circumstances). This is equivalent to more than 50% first-time volunteer donors than registered at the GRC blood donation centers (320,370 first-time volunteer donors in 2003).

This means that a total of approximately 500,000 new volunteer donors for whole blood would have to be recruited in the first 12 months after introducing a deferral based on transfusion history. There are currently 2.2 million active donors in the Federal Republic of Germany. Just under one fourth of these donors will thus have to be replaced.
The total number of additional first-time donors required for permanently compensating for the 4% of donors can be estimated as follows: approximately three fourths of the first-time volunteer donors are suitable as donors. Out of these, only about one third will donate blood for the second time (it is assumed here that they will become permanent donors). Based on this assumption \( \frac{3/4}{3} = 0.25 \), a quarter of the first-time volunteer donors will become permanent donors. Consequently, 4 first-time volunteer donors would have to be recruited for one excluded permanent donor.

If the shortage of donors corresponds to 163,039 whole blood donations originating from 81,520 permanent donors, a total of more than 326,000 additional first-time volunteer donors will have to be recruited in order to permanently compensate for the shortage.

In this model calculation, the only temporary deferred donors who donate again in the same year are not taken into account due to a lack of figures available. Likewise, the possibly deviating donor frequency from the StKB is not taken into account. However, other factors which would negatively influence the figures are not included, e.g.:

- ‘Old’ multiple donors at the GRC blood donation centers donate more than twice a year on average.
- Transfusion recipients donate more (and thus more frequently?) than donors who have not received a blood transfusion (survey by Institute for Transfusion Medicine at Munster, GRC Blood Donation Center West).
- Motivated donors who were themselves dependent on transfusions in the past often recruit new donors among relatives and friends, thanks to their own personal experience. If this way of recruiting donors was lost, we would experience further losses which are not currently quantifiable.
- Newly recruited permanent donors give blood for less than twice a year (survey by GRC Blood Donation Center West).

The above figures are therefore based on a conservative estimate.

**Apheresis Platelet Concentrates**

Based on the assumption that multiple apheresis platelet donors undergo 1.5 apheresis platelet donations 12 times a year (combination of single, double, seldom triple apheresis), the 206,680 apheresis concentrates collected in 2003 originated from 11,482 donors.

If the deferral rate was 4% of these donors (459 donors) the shortage could be calculated as follows:

\[ 459 \times 1.5 \times 12, \text{ corresponding to } 8,262 \text{ apheresis platelet concentrates.} \]

If the calculation described for whole blood donations was used as a basis, 1,800 new donors would have to be recruited. The recruitment effort required for compensation, however, is considerably higher than for whole blood donations since, due to the extreme polymorphism of the HLA system, a multiple of 1,800 donors must be recruited and typed to replace the ‘HLA pattern lost’.

**Pooled Platelet Concentrates**

Since the number of used pooled platelet concentrates could still be manufactured if 4% of the donations were lost, no effects would result here.

**FFP / Plasma for Industrial Processing**

It is difficult to assess to what extent the demand for plasma would no longer be met due to the indications for FFP, which have become stricter, and the decreasing requirement for plasma on the part of industry. However, major shortages are not expected in the case of FFP since the portion of FFP manufactured from whole blood can be increased and permanent donors deferred due to transfusion history could partly be recruited as plasmapheresis donors for plasma for fractionation, if required. There is, however, a possibly counteracting trend to shut down industrial plasmapheresis centers in Germany.

**Appendix (F) Glossary**

<table>
<thead>
<tr>
<th>Term</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>AFSSAPS</td>
<td>Agence Française de Sécurité Sanitaire des Produits de Santé; French medicinal products authority</td>
</tr>
<tr>
<td>A-PC</td>
<td>Platelet concentrate obtained by apheresis</td>
</tr>
<tr>
<td>BSD</td>
<td>Blood donation service (Blutspendedienst)</td>
</tr>
<tr>
<td>BSE</td>
<td>Bovine spongiform encephalopathy; degenerative neurological disease in cattle caused by prions</td>
</tr>
<tr>
<td>CDI</td>
<td>Conformation-dependent immunoassay</td>
</tr>
<tr>
<td>CJD</td>
<td>Creutzfeldt-Jakob disease; TSE disease in humans; transmissible via medicinal products (iatrogenic) or occurring sporadically</td>
</tr>
<tr>
<td>Codon</td>
<td>Sequence of three nucleotides of a gene that specify a particular amino acid of a protein</td>
</tr>
<tr>
<td>DNA</td>
<td>Deoxyribonucleic acid (material of the genome (chromosomes))</td>
</tr>
<tr>
<td>DRK</td>
<td>German Red Cross (Deutsches Rotes Kreuz)</td>
</tr>
<tr>
<td>Dura mater</td>
<td>Tough outer covering of the central nervous system</td>
</tr>
<tr>
<td>EDF</td>
<td>Erythroid differentiation factor</td>
</tr>
<tr>
<td>FFP</td>
<td>‘Fresh frozen plasma’; plasma for transfusion</td>
</tr>
<tr>
<td>GBR</td>
<td>‘Geographical BSE risk’: classification of countries into one of four risk classes (GBR I – IV) by the Scientific Steering Committee of the European Commission</td>
</tr>
<tr>
<td>GRC</td>
<td>German Red Cross (Deutsches Rotes Kreuz)</td>
</tr>
<tr>
<td>GSS</td>
<td>Gerstmann-Sträussler-Scheinker syndrome; a human TSE</td>
</tr>
<tr>
<td>HBV</td>
<td>Hepatitis B virus</td>
</tr>
<tr>
<td>HCV</td>
<td>Hepatitis C virus</td>
</tr>
<tr>
<td>Heterozygous</td>
<td>Different copies (alleles) of a gene in the double (diploid) chromosome set of an individual</td>
</tr>
<tr>
<td>HIV</td>
<td>Human immunodeficiency virus (agent of AIDS)</td>
</tr>
<tr>
<td>Homozygous</td>
<td>Identical copies (alleles) of a gene in the double (diploid) chromosome set of an individual</td>
</tr>
<tr>
<td>i. c.</td>
<td>Intracerebral</td>
</tr>
<tr>
<td>IU</td>
<td>Infectious unit</td>
</tr>
<tr>
<td>i. v.</td>
<td>Intravenous</td>
</tr>
<tr>
<td>Kuru</td>
<td>A human TSE; caused by cannibalism (consumption of deceased persons)</td>
</tr>
<tr>
<td>Symbol</td>
<td>Definition</td>
</tr>
<tr>
<td>--------</td>
<td>------------</td>
</tr>
<tr>
<td>PrPc</td>
<td>Cellular, non-pathogenic form of the prion protein (c = cellular)</td>
</tr>
<tr>
<td>PrPSc</td>
<td>Pathogenic form of the prion protein (Sc = Scrapie)</td>
</tr>
<tr>
<td>O-P</td>
<td>Quarantined plasma</td>
</tr>
<tr>
<td>RBCC</td>
<td>Red blood cell concentrate</td>
</tr>
<tr>
<td>RNA</td>
<td>Ribonucleic acid; among other things: expressed state of a gene</td>
</tr>
<tr>
<td>SCMPMD</td>
<td>Scientific Committee on Medicinal Products and Medical Devices of the European Commission</td>
</tr>
<tr>
<td>sCJD</td>
<td>Sporadic Creutzfeldt-Jakob disease</td>
</tr>
<tr>
<td>Scrapie</td>
<td>TSE disease in sheep</td>
</tr>
<tr>
<td>SD-P</td>
<td>Solvent/detergent-treated plasma</td>
</tr>
<tr>
<td>SRM</td>
<td>Specified risk material; bovine materials in which the BSE agent can be detected in high concentrations (brain, spinal cord, etc.)</td>
</tr>
</tbody>
</table>

**References**


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