but—as evidenced by patient 1, who presented with jaundice, hypoglycemia, and lactic acidosis—it is one with potentially devastating metabolic consequences.

Consistent with the nonsequestering nature of *P. knowlesi*, we did not observe significant neurologic sequelae except in patient 8, who had evidence of a stroke in the context of preexisting cerebrovascular risk. In addition, in contrast with the group of patients with *P. falciparum* infection, the group of patients with *P. knowlesi* included no patients with severe anemia. Both severe anemia and neurologic disturbance have been reported recently as common manifestations of severe vivax malaria [22, 23], but these complications were observed in patients who were younger than those in the present study and in areas of much greater malaria transmission of multiple *Plasmodium* species.

Despite the very high prevalence of thrombocytopenia among our patients with *P. knowlesi* infection (100%, compared with <80% in other human malarias [24–26]), none had a clinically evident coagulopathy. This is consistent with the relative infrequency of bleeding episodes complicating severe falciparum malaria [11], but it is possible that a low platelet count (52,000 platelets/μL) and prolonged prothrombin time (17 sec) contributed to an intracerebral hemorrhage in the patient with knowlesi malaria who died of a probable stroke. The almost invariable presence of thrombocytopenia could facilitate diagnosis of knowlesi malaria. In addition, the significant association between platelet count and *P. knowlesi* parasite density and, in turn, the relationship between parasitemia and markers of severity, could imply that very low platelet counts are of prognostic significance. Such a relationship has been found among African children with falciparum malaria [27].

Although our study included relatively few patients with severe knowlesi malaria, we provide preliminary data relating to the incidence of severe disease. A larger study on the main complications and pathophysiology of knowlesi malaria is in progress, with the aim of establishing specific criteria for severity. It is likely that those for severe falciparum malaria, including neurologic sequelae, severe anemia, and hyperparasitemia [11], may not adequately address the unique biologic properties of *P. knowlesi*. In the case of falciparum malaria, $\geq$250,000 parasites/μL (or 5% parasitized erythrocytes) is conventionally used [11], but thresholds as low as 100,000/μL have been associated with increased mortality and have been used for nonimmune patients [28, 29]. It is therefore important to determine knowlesi-specific markers of disease severity, especially an accurate risk-associated threshold parasitemia.

Our study shows that knowlesi malaria is a significant cause of morbidity in the Kapit Division, extends available data to characterize the spectrum of illness and its clinical course, and confirms our previous observation that life-threatening complications can supervene [2]. Knowlesi malaria is widely distributed in Southeast Asia; it affects mainly people who enter forests or the forest fringe, but the transmission ecology of this potentially serious disease may be changing [30]. Recently, European travellers to Malaysia have received a diagnosis of knowlesi malaria following their return home [31, 32]. The increase in tourism in Southeast Asia may mean that more cases are detected in the future, including in Western countries. Clinicians assessing a patient who has visited an area with known or possible *P. knowlesi* transmission should be aware of the diagnosis, its clinical manifestations, and its course.

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医薬品 研究報告 調査報告書

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研究報告の概要

○英国の匿名扁桃検体中の疾患関連プリオンタンパク質の陽性率:横断的便乗検査

目的:英国の一般集団における疾患関連プリオンタンパク質(PrP(CJD))のより正確な陽性率を確認し、健康への脅威となる変異型クロイツフェルトヤコブ病(vCJD)伝播を低減するための適切な公衆衛生対策に役立てること。

デザイン、調査対象:イングランドおよびスコットランド全域で選択的扁桃摘出術で摘出された匿名扁桃腺検体を対象に、横断的便乗調査を実施した。

主要評価項目:分析原理の異なる2つの酵素免疫法を用いて調べたPrP(CJD)の有無(いずれかの方法で陽性となった場合は、免疫組織化学法または免疫ブロット法による更なる検査を行う)。

結果:2008年9月末までに63,007検体の検査が終了した。このうち12,753例は、vCJDのほとんどが発生した1961～85年生まれの集団であり、19,908例は感染牛肉および加工品によりBSEに曝露した可能性がある1986～95年生まれであった。検査検体のうち、両方の酵素免疫法で明らかな陽性となったものはなかった。1つの検査法で陽性となり、もう一方があいまい(擬陽性)となったのは2検体のみで、残り9検体は両方とも擬陽性結果となった。276検体は、どちらか一方の検査法で初回陽性であった。繰り返し陽性率は15%以下であり、検査法とカットオフの定義により左右された。免疫組織化学法または免疫ブロット法を実施した検体(初回陽性となった276検体すべてを含む)のうちPrP(CJD)陽性となった検体はなかった。

結論:観察された扁桃検体中のPrP(CJD)有病率は、1961～95年生まれの集団では0/32,661(95%信頼区間0～1/13/100万)であった。1961～85年生まれの有病率は0(95%信頼区間0～289/100万)で、過去の虫垂組織の調査(292/100万、95%信頼区間60～853/100万)より低かったが統計的に矛盾はなかった。引き続き扁桃検体を集めて検索することで、特に年長の集団、あるいは他の補完的な大規模な匿名の組織調査(特に剖検組織)の検索により、PrP(CJD)の陽性率の算出精度は更に高まるであろう。

使用上の注意記載状況・その他参考事項等

赤十字アルブミン20
赤十字アルブミン25
赤十字アルブミン20%静注4g/20mL
赤十字アルブミン20%静注10g/50mL
赤十字アルブミン25%静注12.5g/50mL

血液を原料とすることに由来する感染症伝播等

報告企業の意見	今後の対応
英国の匿名扁桃検体中の疾患関連プリオンタンパク質の陽性率調査において、PrP(CJD)陽性が確認された検体はなかったとの報告である。	これまでの疫学研究等では、血液製剤を介して古典的CJD(弧発性、遺伝性および医原性CJD)が伝播するという証拠はない。またCJDの病原因子とされる異常プリオンがアルブミン製剤の製造工程で効果的に除去されるとの報告もあるが、輸血あるいは第Ⅷ因子製剤によりvCJDに感染する可能性が示唆されたことから、今後も引き続き情報の収集に努める。なお、日本赤十字社は、CJD、vCJDの血液を介する感染防止の目的から、献血時に過去の海外渡航歴(旅行及び居住)、CJDの既往歴(本人、血縁者)、hGH製剤投与の有無を確認し、該当するドナーを無期限に献血延期としている。

BMJ

RESEARCH

Prevalence of disease related prion protein in anonymous tonsil specimens in Britain: cross sectional opportunistic survey

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ABSTRACT

Objective To establish with improved accuracy the prevalence of disease related prion protein (PrP^{CJD}) in the population of Britain and thereby guide a proportionate public health response to limit the threat of healthcare associated transmission of variant Creutzfeldt-Jakob disease (vCJD).

Design Cross sectional opportunistic survey.

Study samples Anonymised tonsil pairs removed at elective tonsillectomy throughout England and Scotland.

Setting National anonymous tissue archive for England and Scotland.

Main outcome measure Presence of PrP^{CJD} determined by using two enzyme immunoassays based on different analytical principles, with further investigation by immunohistochemistry or immunoblotting of any samples reactive in either assay.

Results Testing of 63 007 samples was completed by the end of September 2008. Of these, 12 753 were from the birth cohort in which most vCJD cases have arisen (1961-85) and 19 908 were from the 1986-95 cohort that would have been also exposed to bovine spongiform encephalopathy through infected meat or meat products. None of the samples tested was unequivocally reactive in both enzyme immunoassays. Only two samples were reactive in one or other enzyme immunoassay and equivocal in the other; and nine samples were equivocally reactive in both enzyme immunoassays. Two hundred and seventy six samples were initially reactive in one or other enzyme immunoassay; the repeat reactivity rate was 15% or less, depending on the enzyme immunoassay and cut-off definition. None of the samples (including all the 276 initially reactive in enzyme immunoassay) that were investigated by immunohistochemistry or immunoblotting was positive for the presence of PrP^{CJD}.

Conclusions The observed prevalence of PrP^{CJD} in tonsils from the 1961-95 combined birth cohort was 0/32 661 with a 95% confidence interval of 0 to 113 per million. In the 1961-85 cohort, the prevalence of zero with a 95% confidence interval of 0 to 289 per million was lower than, but still consistent with, a previous survey of appendix tissue that showed a prevalence of 292 per million with a 95% confidence interval of 60 to 853 per million. Continuing to archive and test tonsil specimens, especially in older birth cohorts, and other complementary large scale anonymous tissue surveys, particularly of post-mortem tissues, will further refine the calculated prevalence of PrP^{CJD}.

INTRODUCTION

Although the risk to the population of Britain of dietary exposure to the bovine spongiform encephalopathy agent that causes variant Creutzfeldt-Jakob disease (vCJD) has been virtually eliminated, the occurrence to date of four cases of vCJD infection resulting from blood transfusion has made real the threat of a secondary epidemic through healthcare associated human to human transmission.[1-4] These cases from blood transfusion have also established the existence of an infective asymptomatic stage in human vCJD. Estimating the prevalence of this asymptomatic infective stage, although technically challenging, is essential to guide a proportionate public health response to reduce the risk of healthcare associated transmission.

Measurement of prevalence in the 1961-85 birth cohort is a priority, given that 138 of the 167 cases of vCJD to date in Britain have been in this group (with 39 cases in the 1961-9 and 99 in the 1970-85 birth cohorts). Data are available from previous analyses of appendix and tonsil specimens for the presence of disease related prion protein (designated PrP^{CJD}) by immunohistochemistry and immunoblotting.[5,6] The first study screened 11 247 appendix specimens and 1427 tonsil specimens by immunohistochemistry and found three positives in the appendixes from the 1961-85 birth cohort, giving a prevalence of 292 (95% confidence interval 60 to 853) per million.[5] A second study found no positives in 2000 tonsil specimens screened by both immunohistochemistry and immunoblotting;[6] half of these tonsils were from patients aged over

9 years and hence in the birth cohort likely to have had dietary exposure to bovine spongiform encephalopathy. Uncertainty about the true prevalence was increased when back calculation using plausible assumptions from the observed clinical vCJD cases suggested a much lower prevalence of sub-clinical vCJD infection than would be predicted from the finding of PrP^{CJD} in three appendixes.[5,7]

The absence of a suitable blood test for PrP^{CJD}, and doubt about the clinical interpretation for a patient of a positive test result from testing any tissue, created major organisational and technical challenges for our large scale prevalence survey of PrP^{CJD}. To facilitate semi-automated enzyme immunoassay screening, we chose anonymised surgically removed tonsil pairs collected prospectively for the study reported here, rather than appendix tissue already archived in paraffin blocks that would have needed more labour intensive and slower immunohistochemical screening. PrP^{CJD} is known to accumulate to relatively high levels in the tonsils of people with vCJD, although, because of the difficulty of identifying such cases, it has not yet been shown to be present pre-clinically.[8,9]

Commercially available enzyme immunoassay kits are routinely used for testing for bovine spongiform encephalopathy, scrapie, and other animal prion diseases; however, when our survey began no validated kits were available for testing human samples for PrP^{CJD}. We therefore issued a formal tender calling for manufacturers to take part in an enzyme immunoassay selection study and to supply suitable kits. The companies that responded were each sent two blinded panels of samples. Two assays, from Microsens and Bio-Rad, were able to detect brain from vCJD cases diluted 10^{-3} and spleen diluted 10^{-2} into tonsil homogenate (Jillian Cooper, personal communication), and we selected these for use in this study. We now report the results of testing of the first 63 007 specimens from the intended collection of 100 000 in a national anonymous tissue archive.

METHODS

Test validation

We obtained unfixed palatine tonsil samples from 32 sheep with scrapie and 10 that were uninfected, as well as aliquots of unfixed frozen tonsil tissue taken at autopsy from six patients who died of vCJD. We prepared 12% homogenates from these and tested them by both enzyme immunoassays after making a dilution series from 10^{-1} to 10^{-5} with negative human tonsil homogenate. We used a panel of 250 human tonsils that had been previously tested and found to be negative by immunoblotting and immunohistochemistry as examples of "true" negative controls.[6]

Survey tissue samples

Paired tonsil samples from people of all ages, and from operations done between January 2004 and September 2008, were collected from hospitals throughout England and Scotland. One tonsil of the pair was collected as fresh tissue chilled to 4C, and the other tonsil was collected in formalin. Tonsils arrived at the study centre an average of 65 (mode 50, median 113) hours after operation. Once transferred to suitable containers, samples were stored either at −80C (fresh tissue) or at room temperature (fixed tissue).

Patients or their carers were given a leaflet explaining the aims of the study and that any result from testing their tonsil could not be traced back to them. An explicit paragraph and tick box to exercise a right to opt out of inclusion in the survey was included in the pre-tonsillectomy consent forms.

Investigatory algorithm

We homogenised a specimen of each tonsil pair and screened it with both enzyme immunoassays. We defined samples as "reactive," "high negative," or "negative" by a calculation based on the optical density readings from enzyme immunoassay for each microtitre plate. A reactive sample was within three standard deviations of the cut-off, and a high negative was within four standard deviations. We further investigated all samples that were initially reactive in either enzyme immunoassay or gave a high negative result in both enzyme immunoassays by immunoblotting and immunohistochemistry. We re-tested any sample that was high negative in one or other enzyme immunoassay by both enzyme immunoassays, and if it gave a reactive or high negative result in either we investigated it further by immunoblotting and immunohistochemistry. On occasion, we repeated immunoblotting tests with the same and with alternative antibodies.

Definition of a positive result

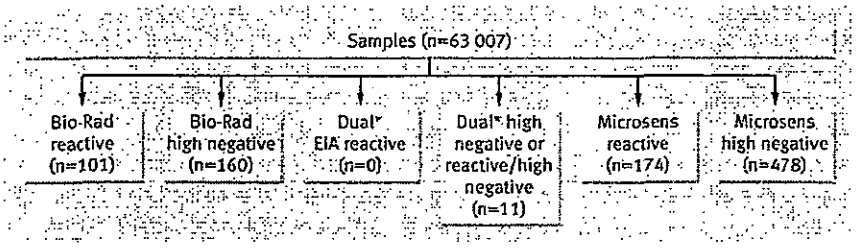
We defined a tonsil positive for PrP^{CJD} as one identified by enzyme immunoassay that was immunohistochemistry positive, had the expected specific protein band pattern in immunoblotting, or both.

RESULTS

Test performance

At a dilution of 10^{-3}, 31 of 32 scrapie sheep samples were reactive in both enzyme immunoassays, and at a 10^{-4} dilution 21were reactive in the Microsens enzyme immunoassay and 16 were reactive in the Bio-Rad enzyme immunoassay. One positive sample was detectable only at a dilution of 10^{-1}. Dilutions of 10^{-2} and 10^{-3} could be detected by immunoblotting.

The six tonsil aliquots from human vCJD cases varied in the amount of lymphoid germinal centre tissue that was present, as judged by visual inspection. Depending on the quality of the tissue, PrP^{CJD} was detectable down to a dilution of 10^{-3} in the Microsens enzyme immunoassay and 10^{-2} in the Bio-Rad enzyme immunoassay (table 1). The amount of PrP^{CJD} detected varied, as judged by the optical density values. This variation may have been due to biological differences in some cases, but an important contributory factor will have been the quality of the available tissue. Immunoblotting of aliquots of the vCJD samples showed that the expected specific band patterns of PrP^{CJD} were



Enzyme immunoassay screening of human tonsil tissue homogenates for PrP^{CJD} *Dual enzyme immunoassay (EIA) reactive samples gave optical density readings above the cut-off classified as "reactive" in both Bio-Rad and Microsens tests; dual high negative or reactive/high negative samples gave optical density readings above the cut-off classified as "high negative" in both Bio-Rad and Microsens tests or was reactive in one and high negative in the other. All EIA reactive samples and most high negative samples were subject to both immunoblotting and immunohistochemistry testing (see text)

detectable. The sensitivities of the enzyme immunoassays were comparable to the immunoblotting results.

Survey specimens collected

Between January 2004 and October 2008, a total of 67 696 tonsil pairs had been archived after collection from 134 hospital trusts throughout England and Scotland. We received forms without tonsil tissue for 1426 patients who objected and 762 in whom clinical pathology examination had been requested. All regions of England contributed samples, and 5651 came from Scotland between January 2006 and September 2008.

We also tested another 2015 anonymous specimens, from tonsillectomies done in the southeast of England between July 2000 and August 2002, of which half were from patients aged over 9 years at operation, and that were untested as part of an earlier survey.[6]

Table 1 | Enzyme immunoassay results on available tonsil tissue from six variant Creutzfeldt-Jakob disease (vCJD) cases (including sample of brain from one case)*: highest dilutions for reported result

	Bio-Rad		Microsens	
Dilution†	Optical density	Interpretation	Optical density	Interpretation
Specimen 1:				
Tonsil 10^{-2}	0.06	High negative	0.12	Reactive
Brain 10^{-3}	0.39	Reactive	0.08	Reactive
Specimen 2:				
Tonsil 10^{-2}	0.04	Negative	0.11	Reactive
Specimen 3:				
Tonsil 10^{-2}	0.06	High negative	0.20	Reactive
Specimen 4:				
Tonsil 10^{-1}	0.04	Negative	0.10	Reactive
Specimen 5:				
Tonsil 10^{-3}	0.04	Negative	0.09	Reactive
Specimen 6:				
Tonsil 10^{-1}	0.13	Reactive	0.21	Reactive

*Three specimens supplied by National CJD Surveillance Unit (including paired tonsil and brain) and three by MRC Prion Unit.
†Dilution from 12% homogenate (10^0); 10^{-1} dilution is therefore equivalent to 0.012 g/ml vCJD tonsil tissue homogenate; as dilution is in negative homogenate, total tissue concentration was 0.12 g/ml for all samples tested.

Enzyme immunoassay screening results

By the end of September 2008, we had screened 63 007 samples with both enzyme immunoassays and, where indicated, completed investigatory testing (figure).

In one or other of the enzyme immunoassays, 276 samples gave an optical density defined as reactive and 638 were classed as high negative (figure). To define the repeat reactivity rate by enzyme immunoassay, we retested 487 reactive and high negative samples by enzyme immunoassay at the beginning of the project, before immunohistochemistry and immunoblotting confirmatory testing. The repeat reactivity rate was 15% (7/48) for the initially reactive samples and 3.5% (4/116) for the initially high negative samples in the Bio-Rad enzyme immunoassay. The equivalent figures for the Microsens enzyme immunoassay were 12% (7/60) and 10% (26/263). All initially reactive samples and any initially high negative samples that gave a repeat reactive or high negative result enzyme immunoassay were subject to immunohistochemistry and immunoblotting confirmatory testing. Any samples that were initially reactive or high negative but which were not repeat tested by enzyme immunoassay went directly for immunohistochemistry and immunoblotting (figure).

No samples were clearly reactive in both enzyme immunoassays. One was reactive by Microsens and high negative by Bio-Rad, and another was reactive by Bio-Rad and high negative by Microsens. Nine were high negative by both the Microsens and Bio-Rad enzyme immunoassays. Seven of these 11 samples were methionine homozygote at codon 129 of the prion protein gene (*PRNP*) and four were heterozygote; only four (three homozygote and one heterozygote) were from people born before 1996 and therefore likely to have had dietary exposure to bovine spongiform encephalopathy.

Immunoblotting results

We demonstrated satisfactory immunoblotting performance, using two different protocols in two separate laboratories, by testing the tonsil tissue taken at autopsy from vCJD patients, as well as by spiking experiments using scrapie sheep tonsil tissue, scrapie infected hamster brain, and human vCJD brain tissue.

None of the survey sub-sample investigated by immunoblotting gave a protein banding pattern consistent with the presence of PrP^{CJD}. Some samples that showed a single band, which was not consistent with any expected pattern, were re-tested by immunoblotting either with the same antibody or with different antibodies, including 3F4 and a secondary antibody designed to reveal non-specific antibody interactions. Only one sample still showed a single immunoblotting band; it was methionine homozygote at codon 129 and from a patient in the 1986-90 birth cohort, and it was negative by immunohistochemistry.

Immunohistochemistry results

More than 800 tonsils, selected on the basis of the enzyme immunoassay results, have been investigated

by immunohistochemistry in one or other of two experienced laboratories, and none was scored positive for PrP^{CJD}.

Prevalence estimates

Overall, 32 661 (52%) of the 63 007 samples tested came from people born in 1995 or earlier who were alive at the time when bovine spongiform encephalopathy contaminated meat was being consumed (table 2). The observed prevalence of PrP^{CJD} in this group was zero (95% confidence interval 0 to 113 per million). Combining the 1986-90 and 1991-5 cohorts gave a prevalence of zero with an upper 95% confidence limit of 185 per million. The prevalence in the combined 1996-2000 and 2001-7 unexposed cohorts was also zero with an upper 95% confidence limit of 122 per million.

Although the zero per million prevalence seen in the 1961-85 cohort (upper 95% confidence limit 289 per million) was different from the 292 per million (95% confidence interval 60 to 853 per million) found in the earlier survey of appendix tissue,[5] the 95% confidence intervals for both surveys overlapped (a formal comparison of the prevalence estimates gives a P value of 0.09).

DISCUSSION

Initial results from testing the tonsil specimens in a national anonymous tissue archive have shown the prevalence of PrP^{CJD} to be zero in 63 007 overall and zero in 12 753 in the birth cohort in Britain in which most cases of vCJD have occurred. Interpretation of this finding, and of the difference between it and the earlier survey of appendix tissue, depends critically on three factors: the sensitivity of the test system chosen to screen the tonsil specimens, the representativeness of the sample specimens of the people most vulnerable to vCJD disease, and the natural history of the infectivity of bovine spongiform encephalopathy in individual patients, particularly the time when PrP^{CJD} first appears pre-clinically in tonsil compared with appendix tissue and how long it persists.

Table 2 | Prevalence of disease related prion protein (PrP^{CJD}) in Britain by birth cohort (positive/total; rate per million with 95% confidence intervals*)

Birth cohort	Current (2004-September 2008) national tissue survey: tonsils	Earlier† (1995-9) national tissue survey	
		Appendices	Tonsils
1940 and before	NA	NA	0/225
1941-60	NA	0/573	0/266
1961-85	0/12 753; 0 (0 to 289)	3/10 278; 292 (60 to 853)	0/694
1986-90	0/9 564; 0 (0 to 386)	0/396	0/119
1991-5	0/10 344; 0 (0 to 357)	NA	0/106
1996-2000	0/15 708; 0 (0 to 253)	NA	0/17
2001-7	0/14 638; 0 (0 to 252)	NA	NA
Total	0/63 007; 0 (0 to 59)	3/11 247; 267 (55 to 779)	0/1 427; 0 (0 to 2 582)

NA=not available.
*95% confidence interval calculated only when denominator exceeds 1000.
†Data from separate tissue survey of 2000 tonsils (July 2000-August 2002) in southeast England (including London)[6] not included.

Test sensitivity

Three experiments investigated the sensitivity of the enzyme immunoassays. The first was the enzyme immunoassay selection study, the second was the interrogation of the enzyme immunoassays with tonsil tissue from sheep with scrapie, and the third was the use of tonsil tissue from patients who died from vCJD. Overall, these indicated that the Microsens enzyme immunoassay was more sensitive than the Bio-Rad enzyme immunoassay for detection of PrP^{CJD} in lymphatic tissue. The most sensitive detection was by the Microsens enzyme immunoassay with a sample containing 12 µg vCJD tonsil tissue; the equivalent for the Bio-Rad enzyme immunoassay was 480 µg vCJD tonsil tissue (table 1). When used for screening, 12 000 µg tonsil tissue was applied to the Microsens enzyme immunoassay and 48 000 µg to the Bio-Rad enzyme immunoassay. Therefore, the two enzyme immunoassays should have been sufficiently sensitive to detect PrP^{CJD} in tonsils from asymptomatic people incubating vCJD if levels of PrP^{CJD} were a 10th to a 1000th of those in patients with symptoms.

The dual enzyme immunoassay tonsil screening protocol may be at least as sensitive as any other large scale testing for abnormal prion protein that could have been used. The enzyme immunoassays use different test principles and antibodies, perhaps reinforcing the sensitivity of each. Reading of the results was automated, and we used a range of controls on each 96 well plate of tests. We deemed the use of a single enzyme immunoassay cut-off value as commonly applied to screen a population with many positives to be inappropriate, as this particular set of samples was expected (and found) to be overwhelmingly negative. Therefore, we calculated the cut-off value for each plate individually, and this method almost doubled the number of specimens that were selected for further investigation by immunoblotting and immunohistochemistry.

Several reasons exist why a specimen could have given a false high (reactive or high negative) optical density reading in either or both enzyme immunoassays: inadequate proteinase K digestion of PrP^{C} (the normal cellular form of PrP) for the Bio-Rad enzyme immunoassay, inadequate removal of PrP^{C} bound to the capture polyanion for the Microsens enzyme immunoassay, non-specific antibody interactions owing to the high antibody concentration in tonsil tissue, and poor sample quality or technical failures. Therefore, applying more specific immunoblotting and immunohistochemistry tests to confirm whether PrP^{CJD} was present was essential.

In comparison with immunohistochemistry, the volume of tonsil tissue screened by enzyme immunoassay was relatively large. Immunohistochemistry on appendix tissue may also be less specific than immunoblotting, so that prevalence estimated by immunohistochemistry screening may tend to overestimate the true situation.[9] However, to tackle the lingering uncertainty that screening immunohistochemistry might be more sensitive than dual enzyme immunoassay

screening, a further study to re-test 10 000 of the archived tonsils by immunohistochemistry has been commissioned. These 10 000 samples comprise those from patients in the 1961-85 birth cohort, as well as any samples that gave optical density readings above the cut-offs in either of the two enzyme immunoassays. The results from this major undertaking should be available some time during 2009.

Two of the three positive samples in the retrospective immunohistochemistry study of appendix tissue were valine homozygous at codon 129 of *PRNP*.[5 10] Therefore, we can be confident that the antibodies used in our immunohistochemistry analysis would have showed PrP^{CJD} in a valine homozygote if it was present. The antibodies used in the enzyme immunoassay and immunoblotting would similarly be likely to detect PrP^{CJD} in a valine homozygote and, by extension, PrP^{CJD} in a heterozygote. Although the immunoblotting profiles of valine homozygote and heterozygote vCJD are unknown, they may be expected to consist of three or four glycoforms.[11] The immunoblotting profile of the spleen in a case of asymptomatic vCJD infection in a heterozygote patient showed similarities to that in clinical vCJD spleen samples in methionine homozygote patients, with a predominance of the diglycosylated band.[2] We did not observe by immunoblotting any pattern similar to any recognised profiles in sporadic CJD or vCJD.[12-16] The only repeatedly anomalous immunoblotting pattern seen was of a single immunoblotting band in an immunohistochemistry negative sample, which was methionine homozygote at codon 129 of *PRNP*.

Representativeness of sample

The age and sex characteristics of the samples in our study reflected the current age and sex distribution of people having tonsillectomy: 72% of those born in 1995 or earlier in our survey were female, compared with 48% of those born since 1995. Although only 44% of vCJD cases to date have been in women, we do not think that the predominance of females in our older sample of tonsils could have biased our findings with respect to prevalence of PrP^{CJD}.

Given the very strong association between PrP^{CJD} and people who are homozygous for methionine at *PRNP* codon 129,[5] it is important to note that our sample was likely to have been representative of this genetic susceptibility: an analysis of 466 of the tonsils in our survey showed 47% to be methionine homozygotes at codon 129, consistent with what was expected.[10 17-20] Therefore, of the 32 661 tonsils tested from people born before 1996, approximately 15 351 (47%) would have been from methionine homozygotes.

Several differences must be considered when comparing results between surveys. First and foremost is that previously appendix tissues were screened by immunohistochemistry, whereas we screened tonsil tissue by enzyme immunoassay. Secondly, an average of 10 years elapsed between when the previous large sample from the 1961-85 birth cohort had their appendixes removed (during 1995-9) until our sample had their tonsils removed (mostly in 2006-7)—10 years during which abnormal prion protein levels might be expected to have increased rather than diminished. Within this birth cohort, however, the average age of appendicectomy was estimated to be four years older than the average age of tonsillectomy, so the average duration of the opportunity for PrP^{CJD} to increase between the appendicectomy samples and the tonsillectomy samples would have been about six years. On the other hand, the relatively older appendix sample that was collected earlier may conceivably have contained a wave of infectivity in the 1961-85 cohort of the British population that was not present in the younger tonsil group that was sampled later.

Detailed information on previous operative history was sought on every vCJD case diagnosed in Britain. Seventeen of 167 patients were reported to have had tonsillectomy; 14 of these were in the 1961-85 birth cohort, and the remaining three were in the pre-1960 birth cohort. None was likely to have had specimens included in this or the earlier tonsil survey (Hester Ward, personal communication).[6]

Natural history

While PrP^{CJD} has been found consistently by immunoblotting and immunohistochemistry in tonsil tissue from patients with vCJD,[8 9 21-24] PrP^{CJD} in a tonsil from an asymptomatic person has yet to be reported. Given, however, that tonsillar tissue has been shown to accumulate PrP^{Sc} before the onset of clinical disease in non-human primates and well before the onset of clinical disease in sheep experimentally infected orally with bovine spongiform encephalopathy,[25 26] we considered tonsil tissue to be a reliable substrate for a survey of prevalence in humans. Also, the use of fresh tonsil tissue allowed more comprehensive laboratory testing, if necessary, after the initial screening assays.

PrP^{CJD} has been observed to accumulate in appendix tissue in vCJD (19/20 positive/tested)[9 27 28] and, in two cases, before symptoms developed.[29 30] However, data on the timing of the appearance of PrP^{CJD} in different peripheral lymphoreticular tissues during the prolonged incubation period of vCJD are sparse. The rate of accumulation of PrP^{CJD} in tonsil and appendix tissue could differ such that the findings of surveys of appendix and tonsil tissues would also differ. The positive samples found in the appendix survey presumably came from people who were infected a relatively short time earlier, during the peak of the bovine spongiform encephalopathy epidemic.[5] Moreover, should the incubation period for prion disease be considerably longer in people with different genotypes, uncertainty about the timing of the appearance of detectable PrP^{CJD} in these will increase, with concomitant implications for the interpretation of results of PrP^{CJD} prevalence surveys.

Animal experiments have shown that high infectivity, and indeed disease, can be present in the absence of detectable proteinase K resistant PrP^{Sc}.[31] The extent to

WHAT IS ALREADY KNOWN ON THIS TOPIC

Statistical back calculation based on cases of vCJD to 2004 has given estimates of between 10 and 190 further clinical cases over the next few decades

A study of archived appendix and tonsil tissues found a prevalence of lymphoreticular accumulation of pathogenic prion protein consistent with the existence of between 520 and 13 000 sub-clinical cases

Therefore, a discrepancy exists between estimates, which needs to be resolved to ensure that proportionate public health measures are implemented

WHAT THIS STUDY ADDS

Testing of tissue from more than 63 000 tonsils, of which 12 763 were from the 1961-85 birth cohort, has not shown evidence for the presence of the pathogenic form of the prion protein

The prevalence of sub-clinical vCJD infection in Britain may be lower than that given by previous estimates, with an upper limit of 289 per million in the 1961-85 birth cohort

which this observation can be generalised is, however, unclear, as PrP^{CJD} has been shown to be present in the lymphoid tissues of all vCJD patients tested.[9 27] If other, more reliable, indicators of vCJD become available, screening the existing samples with tests for these markers, and thereby determining whether any vCJD positives have been missed by looking only for PrP^{CJD}, may be possible.

Data from animal experiments also show "clearance" of abnormal prion protein after inoculation.[31 32] Therefore, the abnormal prion protein found in the earlier survey of appendix tissue may conceivably have been transient and eventually cleared without leading to disease, so that the appendix survey result would not have been replicated by the later tonsil survey.

Conclusion

We tested more than 32 000 tonsils from people in the age range most exposed to meat contaminated with bovine spongiform encephalopathy, and believed to be asymptomatic when sampled, for disease related prion protein. Using two sensitive enzyme immunoassays, with selective application of specific immunoblotting and immunohistochemistry techniques, we found no samples positive for PrP^{CJD}, a prevalence of 0 per million (with an upper 95% confidence limit of 113 per million). For the 1961-85 birth cohort, the prevalence of zero with a 95% confidence interval of 0 to 289 per million was lower than, but still consistent with, the earlier study of appendix tissue (60 to 853 per million). A P value of 0.09 applies to the comparison of the two prevalence estimates. These two surveys may not, however, be directly comparable owing to differences in testing methods, tissues sampled, and the time the tissues were removed (typically about 10 years earlier in the previous study). More data are needed through continuing the testing of tonsils from people born before 1996, despite the low frequency of tonsillectomy in older birth cohorts. In addition, creation and testing of other anonymous tissue archives, such as one based on coronial autopsies, or a repeat of the appendix survey on an even larger scale, should provide a larger sample set of the people most exposed to the bovine spongiform encephalopathy agent.[33]

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This study was originally proposed by a Medical Research Council and Department of Health committee. It was overseen by an Expert Advisory Group on the Laboratory Testing Strategy for Large Scale Abnormal Prion Prevalence Studies with a membership of N Andrews, D W G Brown, J P Clewley, J Cooper, R Eglin, E Gadd, O N Gill, M Head, D A Hilton, J W Ironside, G Jackson, C M Kelly, G Mallinson, D Matthews, P Minor (chair), P P Mortimer, N Raven, J R Stephenson, and J D F Wadsworth. Data from this anonymous tonsil survey were discussed in public at meetings of the Spongiform Encephalopathy Advisory Committee (www.seac.gov.uk) in December 2007 and April 2008; and see a statement issued in August 2008 (www.seac.gov.uk/statements/state-cjd-infections.pdf).

Contributors: JPC designed and analysed the laboratory studies and wrote the paper with ONG, who initiated the study and did clinical and epidemiological analyses. CMK recruited hospitals to the study and did epidemiological analyses. NA did statistical and epidemiological analyses. KV organised the National Anonymous Tissue Archive laboratory, tonsil processing, and enzyme immunoassay testing. GM, MK, and RD did the immunoblotting. DAH, PE, JWI, LMcC, and DLR did the immunohistochemistry. JWI provided some of the vCJD clinical tissue used in the work. HEA did the codon 129 genotyping. JPC and ONG are the guarantors.

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Competing interests: None declared.

Ethical approval: The study received ethical approval from the Trent Multi-centre Research Ethics Committee (MREC/03/4/073). None of the participants in the study was subsequently identifiable.

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研究報告の概要

○複数ルートに曝露された患者のvCJD リスク評価算定
変異型クロイツフェルトーヤコブ病(vCJD)を発症しておらず、vCJDとは関係ない疾患により死亡した血友病患者の剖検時に、脾臓よりvCJD異常プリオンタンパク質が検出された。血友病患者または血漿分画製剤の治療を受けた患者に、vCJD異常プリオンタンパク質が見つかったのはこれが初めてである。
患者は、当該患者は、内視鏡手術、赤血球輸血、英国の血漿由来血液凝固第VIII因子製剤頻回投与等、複数のvCJD感染ルートに暴露された。vCJDに関する血液安全性改善のための措置が導入された1999年以前に英国内で供血された血液由来の凝固因子製剤による治療を受けたことが判明している。また、1996年に血漿の供血を行ってから6か月後にvCJDの症状を発現した供血者に由来する血漿から製造された第VIII因子製剤1ロットの投与を受けている。英国の供血者の潜在的なvCJD感染リスク(有病率約1:10000)を考慮すると、患者はvCJD発症ドナーが関連していない第VIII因子製剤によってvCJDに感染した可能性が最も高いと考えられた。

使用上の注意記載状況・その他参考事項等

赤十字アルブミン20
赤十字アルブミン25
赤十字アルブミン20%静注4g/20mL
赤十字アルブミン20%静注10g/50mL
赤十字アルブミン25%静注12.5g/50mL

血液を原料とすることに由来する感染症伝播等

報告企業の意見	今後の対応
変異型クロイツフェルトーヤコブ病(vCJD)を発症しておらず、vCJDとは関係ない疾患により死亡した血友病患者の剖検時に、脾臓よりvCJD異常プリオンタンパク質が検出され、vCJD発症ドナーが関連していない第VIII因子製剤によって感染した可能性が最も高いと考えられたとの報告である。	プリオン病の原因とされる異常プリオンが分画製剤製造工程で効果的に除去されるとの成績と併せて、これまでの疫学研究では如何なるプリオン病も、血漿分画製剤を介して伝播するという証拠は無かった。しかし、原因が特定されていないものの、本報告で初めて、第Ⅷ因子製剤を介してvCJDに感染する可能性が示唆された。引き続きプリオン病に関する新たな知見及び情報を収集するとともに、血漿分画製剤の製造工程における病原因子の除去・不活化技術の向上に努める。なお、日本赤十字社は、CJD、vCJDの血液を介する感染防止の目的から、献血時に過去の海外渡航歴(旅行及び居住)、CJDの既往歴(本人、血縁者)、hGH製剤投与の有無を確認し、該当するドナーを無期限に献血延期としている。

vCJD Risk Assessment Calculations for a Patient with Multiple Routes of Exposure

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5th June 2009

Preface

This paper was developed in response to a request from the CJD Incidents Panel following the finding of abnormal prion protein in the spleen of a patient with haemophilia. Assuming that the abnormal protein represents a marker of vCJD infection, the paper sets the various possible routes through which such infection could have occurred, and considers their relative likelihood in various scenarios. As well as dealing with this specific "incident", the paper sets out a more general methodology for assessing multiple possible infection routes. The analysis was considered by the Panel at its meeting on 20th May 2009, and informed the advice subsequently issued. This version of the paper repeats the analysis presented to the Panel, while giving slightly more background information for other readers, and is placed here for public record.

Introduction

1. This paper offers an analysis of the recent finding of abnormal prion protein in the spleen of a haemophilic. This involves a patient exposed to a large number of potential vCJD infection routes (including multiple blood component transfusions, repeated receipt of UK-sourced fractionated plasma products including some units linked to a donor who later went on to develop clinical vCJD, and several invasive biopsies) who was found post mortem to have abnormal prion protein in a spleen sample.

2. If this finding is interpreted as an instance of asymptomatic vCJD infection, this raises questions as to the operational meaning of the "prevalence" of infection. The discovery of abnormal protein in a single spleen sample was the only positive result after exhaustive investigation of tissues taken at autopsy of an elderly haemophilia patient who died of other causes with no symptoms of vCJD or other neurological condition. All other tissues from this patient tested for the presence of abnormal prion protein – fixed samples of brain, heart, liver, blood vessel, appendix, spleen and lymph node and frozen samples of frontal lobe, occipital lobe, cerebellum, lymph node and 23 other samples from the spleen – were negative. This individual would not have tested "positive" on any of the vCJD prevalence tests conducted so far, and possibly not even in a post mortem spleen survey (depending on the size of spleen sample used). Nor do we know whether someone with this limited distribution of abnormal prion protein would be infective - and if so, by what routes of transmission.

3. For present purposes, however, these issues of interpretation are ignored. We simply assume that the abnormal prion protein found in this patient is a marker for asymptomatic vCJD infection: the task is then to investigate the relative likelihood of the infection having come from the various possible routes. This is done in order to inform discussion by the CJD Incidents Panel ("the Panel") as to the implications of the finding, and in particular whether the new evidence warrants any change to the "at risk" status of any individuals or groups.

4. The ideal would be to quantify these likelihoods in a robust way. However, this is not possible due to the multiple uncertainties involved. These are well-rehearsed. We do not know the prevalence of infectious donors – and in this instance, some of the potential routes are dependent on prevalence while others are not, so the relativities change. The probability of an infected blood component transmitting infection is uncertain - though on the precautionary approach adopted by the Panel, it is presumed to be substantial. The risks of an implicated plasma derivatives transmitting infection are even more uncertain. However, they can be estimated using methods suggested in an existing assessment by independent consultants DNV (DNV, 2003), which have been used in drawing up Panel recommendations to date. These calculations have also been regarded as "precautionary", i.e. giving a pessimistic view of the levels of infectivity likely to be present.

5. Given these unknowns, we make no attempt at definitive probability calculations, though illustrative examples are provided. Instead, we concentrate on the more limited task of determining whether different groups in the complex chain of contacts associated with the index patient can be robustly placed under or above

the additional 1% (over the UK population risk derived from consumption of beef and beef products) "risk threshold" used by the CJD Incidents Panel to trigger decisions on notification of increased risk status. We also consider the wider implications for groups that are or might be classed as "at risk". Although the analysis does throw some light on these questions, it also highlights some conundrums for our understanding of vCJD prevalence and transmissibility.

Summary of findings

6. Specifically, we conclude that on the evidence available:

(i) **The chance of the patient having been infected via an endoscopic procedure is very small,** probably comparable to that of having been infected via primary (dietary) exposure. The potential risk associated with the endoscopies can be disregarded in assessing the risks associated with the possible blood-borne transmission routes, and no specific action is called for with regard to other patients on whom those endoscopes may have been used.

(ii) Comparing the blood-borne routes, **the patient is much more likely to have been infected through receipt of plasma products, rather than any of the 14 units of red cells known to have been received.** The implied risk of each of these 14 donors being infected appears to lie below the 1% threshold that would trigger "at risk" status.

(iii) Given the large pool sizes involved (of the order of 20,000 donations per pool), **the risk differential between "implicated" and "non-implicated" batches of blood product is not marked.** Unless the prevalence of infection is very low, there is a strong possibility of *any* given batch of blood products prepared from large pools sourced from UK donors in the period 1980-2001 containing at least one infected donation. This reinforces the logic of the CJD Incidents Panel's 2004 decision to consider all haemophilia and blood disorder patents exposed to such UK-sourced plasma products as an "at risk" group. There is no strong case for differentiating between sub-groups.

(iv) Given the precautionary assumptions in the DNV risk assessment, any patient exposed to substantial quantities of UK plasma product (as this haemophilia patient was) would almost certainly have received a substantial infective dose, *whether or not* any of the batches were "implicated" (i.e. traceable to a donor who later went on to develop clinical vCJD). In fact, this patient may have been **more likely to have been infected by receipt of large quantities of "non-implicated" plasma, than by the smaller quantities of "implicated".**

(v) The lack of any clinical vCJD cases to date amongst patients with haemophilia may suggest that the DNV infectivity scenario is overly-pessimistic. Risk assessments carried out elsewhere assume that a greater proportion of the infectivity would be removed during the manufacturing processes. This raises issues beyond the scope of this paper. Nevertheless, we have re-run the analysis using a markedly lower infectivity assumption with regard to plasma products, and the conclusions listed in (ii) – (iv) above still hold.

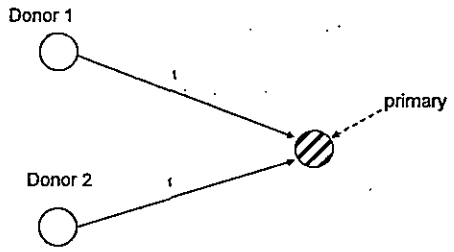
Method

7. The following analysis starts from the "reverse risk assessment" previously used by the Panel to assess the implied risks of donors to vCJD clinical cases being infected (DH, 2005a; Bennett, Dobra and Gronlund, 2006), and extends it to deal with this much more complex incident. We start with a simple example and then build up the analysis step-by-step. This is both to demonstrate how the conclusions are reached in this case, and to show how the same approach can be used to handle other complex incidents that may arise.

Example 1

8. We therefore start with a simple incident as shown in Figure 1(a). Here, a patient has received two single-unit Red Cell transfusions, one from each of two donors. The recipient goes on to develop vCJD, and the timing of the transfusions does not rule either of the donors out as the route of infection. What is the chance of each of these donors carrying vCJD infection?

Figure 1 (a) Two component donors, neither known to be infected



9. The answer to this depends primarily on the chance of transmission occurring *if* one of the donors were to be infected – i.e. the transmission probability, t. By definition, this lies between 0 and 1: if t = 1, transmission would be certain. In that case, and all else being equal[1], the patient's disease would be equally likely to have come from primary infection, or from either of the two donors having been infected. So by implication, each donor would have a 1 in 3 chance of being

[1] "All else being equal" essentially means that there is no prior reason to suppose that donors or recipient were particularly likely or unlikely to have been infected with vCJD, e.g. through "high risk" surgery, or conversely not having lived in the UK during years of high BSE exposure.

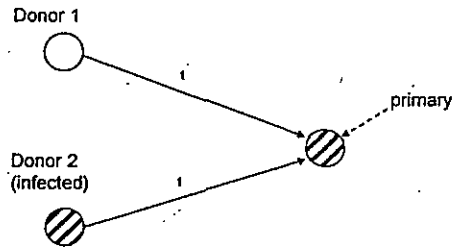
infective.[2] More generally, if there are n donors, the chance of each being infective would be 1/(n+1).

10. The implied risks to the donors clearly diminish if t <1. However, the CJD Incidents Panel has used a precautionary approach, concentrating on scenarios in which t is at least 0.5. With t in this range, the implied risk to donors remains high unless the number of donors to the vCJD case is large. For example, if t = 0.5, then with two donors the chance of either being infected would be roughly 0.25. Note that none of these calculations depend on the underlying prevalence of infection, provided this is the same for donors and recipients.

Example 2

11. The situation would clearly be very different if one of the donors was later diagnosed with vCJD, as in Figure 1(b).

Figure 1 (b) Two component donors, one known to be infected



This creates a marked asymmetry between the infection routes, dependent on the prevalence of infection in the donor population. Whilst Donor 2 is now known to be infected, Donor 1's prior probability of infection is simply the prevalence of infection (p), unknown but assumed to be small. This situation provides an exemplar for analyses in which some routes are prevalence-dependent and others are not.

Let:

P(D1) be the probability of the recipient's infection having come via Donor 1

[2] The arguments expressed here can be expressed more formally using Bayes' Theorem to update probabilities in the light of new information. However, this is presentationally more clumsy, especially in the more complex examples considered below.

P(D2) be that of the infection having come via Donor 2

and P(prim) be the probability of the recipient having a primary infection

- For simplicity, suppose that the chance of the patient being infected by more than one route is negligible. Then (given that infection has occurred) P(D1), P(D2) and P(prim) must add up to 1.

- Furthermore, the "balance" between the three probabilities will be governed by t and p. Specifically:
    - P(D1) will be proportional to both p (prevalence of infection) and t (transmission probability)
    - P(D2) will only be proportional to t
    - and P(prim) will only be proportional to p

12. Provided p is small (e.g. 1/4,000 or 1/10,000) and t is not, P(D2) will be *much* larger than either of the other two probabilities. To a very close approximation, P(D2) = 1 and P(D1) and P(prim) are zero. We can be virtually certain that the infection came from Donor 2. In practical terms, this new information about Donor 2 means that Donor 1 need not be considered as "at risk" according to CJD Incidents Panel criteria.

Example 3

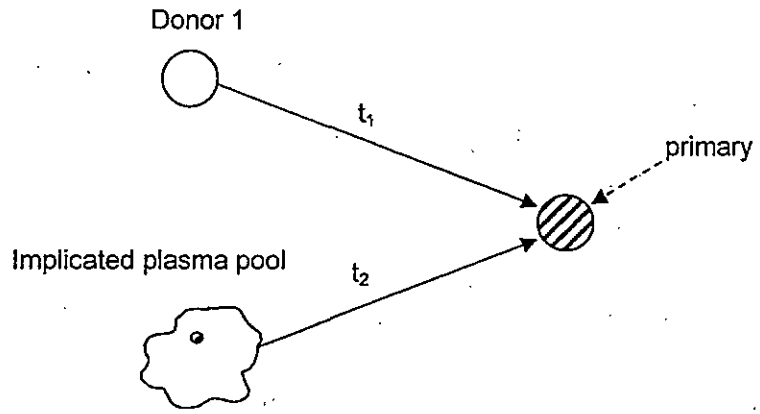
13. In the last two examples, the two secondary routes had the same transmission probability, t. But suppose now that there are routes with different values of t – e.g. transfusion of blood components and receipt of fractionated blood products. Figure 2 below shows a situation in which the calculations need to balance two contrasting secondary routes:

    - a blood component transfusion, associated with a high transmission probability (t_1) *if* the donor (D1) is infected, but with no reason to believe that this is the case, and
    - a plasma product pool with a contributing donor (D2) now known to be infected , but with a low transmission probability (t_2)

    As before, the three probabilities P(D1), P(D2) and P(prim) must add up to 1, and now:

    - P(D1) will be proportional to p and t_1
    - P(D2) will be proportional to t_2
    - and P(prim) will be proportional to p

Figure 2: One component donor, not known to be infected: plasma pool, containing an implicated donation



14. To illustrate numerically, suppose p is 10^{-4} i.e. prevalence of infection is 1 in 10,000, that $t_1 = 1$ and $t_2 = 10^{-3}$ (that is, transmission via the product pool is less efficient than via the transfused component by a factor of 1,000).

In that case, it can be shown that:

$$P(D1) = 1/12 \qquad P(D2) = 10/12 \qquad \text{and} \qquad P(prim) = 1/12$$

The infected plasma pool is thus clearly the most likely transmission route, by a factor of 10 over each of the other two possibilities.

15. The principles used to analyse these simple cases are now extended to consider the case of the haemophilic patient with a finding of abnormal prion protein in the spleen.

Analysis

16. Potential secondary transmission routes in this instance consisted of the following (where an "implicated" donor means one for which there is now evidence of having been infected with vCJD):

- 5 invasive endoscopic procedures (biopsies) and a larger number of endoscopies without biopsy.
- exposure to 14 units of Red Cells, each from different ("non-implicated") donors
- exposure to just over 9,000 units of Factor VIII made from two plasma pools with an "implicated" contributing donor (8,025 units from one batch and 1,000 from the other)

- exposure to many other units of UK-sourced pooled products, including nearly 400,000 units of Factor VIII, with no *known* links to "implicated" donors

To simplify the subsequent discussion, we consider the relative risks from each of these routes in turn.

Transmission risks from the endoscopies

17. vCJD transmission risks from endoscopy have been examined by an ACDP TSE WG subgroup, informed by an outline risk assessment. It is important to appreciate that these procedures involve a very small instrument (head) being passed down a very long, thin, channel. The possible "mechanics" of infection therefore differs from other surgical procedures. The group considered that any significant risk of onward transfer of infective material to a receptive site would require the procedure to be invasive, as distinct from examinations that involve the instrument sliding against the wall of the gut. On that argument, the relative risk from endoscopic procedures *not* involving biopsy would be negligible.

18. So concentrating on procedures involving biopsy, the question arises of whether the heads used would have been single-use. This would reduce the transmission risks considerably, but not eliminate them (due to the possibility of the new head being contaminated on its way down the endoscopy channel. Although we do not know whether the heads involved in these procedures were single-use, let us suppose they were not.

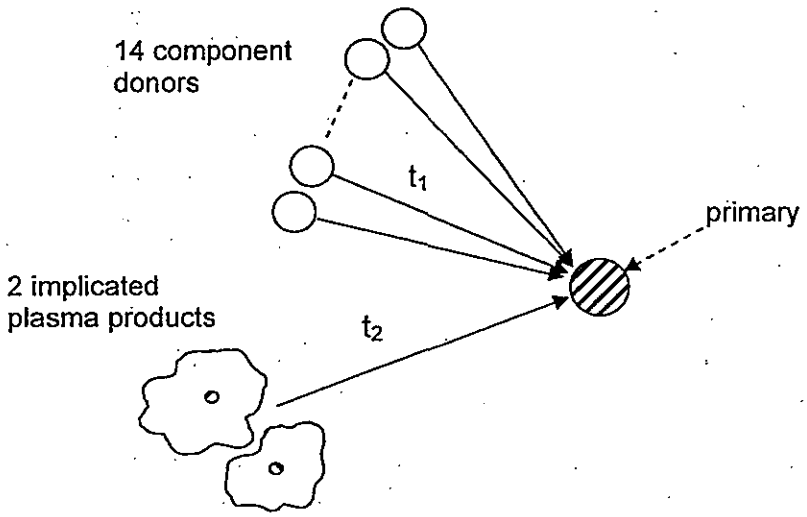
19. For endoscopy with re-useable heads, the best existing analogy is with the current surgical risk assessment as applied to procedures encountering lymphoid tissue. Depending on assumptions on the efficacy of decontamination, the "standard" model suggests that indefinite re-use of a set of instruments might cause 1 – 10 secondary infections per operation on an infective patient. The infection risk to a random patient resulting from all previous re-uses of the instruments would be in the same range multiplied by the prevalence of infection (p). However, the surgical model considers the transmission risks from a set of 20 instruments, rather than just one (very small) biopsy head. For the latter, it therefore seems reasonable to reduce the estimated risk by a factor of at least 10. Even on pessimistic assumptions, therefore, the risk of infection from a "random" biopsy would be in the range (0.1 – 1)p. In other words, the chance of the patient being infected via any of 5 such biopsies would be similar to the risk of having been infected through the "primary" route of dietary exposure.

20. As will be seen below, the chance of this particular patient having been infected by the primary route are very small (in all scenarios) as compared to that of infection through a blood-borne route. On the above argument, the same applies to the endoscopic route. For simplicity, this route will therefore be disregarded in the following calculations. It should be noted that even if the risks of transmission via endoscopy were much greater than suggested here, the only effect on subsequent calculations would be to reduce the probabilities associated with all the blood-borne routes slightly.

Blood components and "implicated" plasma products

21. We now consider the relative probability of the patient's infection having come from the implicated plasma products, versus the 14 Red Cell transfusions. As discussed in the "methods" section, we need to balance the greater transmission probability for blood components (Red Cells in this instance) against the existence of an implicated donor contributing to the pooled plasma products. The situation is shown schematically in Figure 3, omitting for now the other "non implicated" plasma products.

Figure 3: 14 component donors, none known to be infected; 2 plasma products, each from a pool containing an implicated donation



22. The key additional variable here is t_2 – the chance of transmission from an implicated pool. This can be quantified using the infectivity assumptions originally generated in DNV's risk assessment (DNV, 2003). As discussed further below, the calculations initially use the more pessimistic of alternative infectivity scenarios considered by DNV.

23. For the present, we also suppose that the *only* infected donation in the plasma pools came from the identified infected donor – though this is reconsidered below. As detailed in the first part of Annex A, calculations then suggest that this one infected donor would have resulted in the Factor VIII received by the patient containing a total infective dose of about 0.2 ID_{50} (0.16 via one pool and 0.05 via the other). Using the simple linear dose-response model that has informed Panel recommendations to date, this implies a transmission probability t_2 of approximately 0.1.

24. We can then use the approach set out before to assign probabilities to the possible infection routes in different scenarios. Table 1 below shows the results, using this value for t_2 and alternatives of 1 and 0.5 for t_1 and 1 in 4,000 and 1 in