CJD関連各種論文等について

資料4－3

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CJDに関する各種論文等（要約）一覧表

資料番号			概要
A. 血漿分画製剤とvCJDについて			
A-1 血液凝固第Ⅷ因子製剤投与歴のある患者脾臓に異常プリオンタンパクが集積していた事例について			
①	英国保健省(2009.6.5)	vCJD Risk Assessment Calculations for a Patient with Multiple Routes of Exposure	後にvCJDを発症した供血者からの血漿が入った原料血漿から製造された第Ⅷ因子を投与された血友病患者がvCJD以外の疾患で死亡し、剖検によって脾臓から異常プリオンタンパクが検出された。脳を含めた他臓器からは検出されなかった。なお、当該患者の脾臓検体24切片の異常プリオンタンパクが陽性であったものは1切片であり、他の23切片は陰性であった。 問題の第Ⅷ因子製剤のロットは、 FHB4547: 26303人のプールから製造され、DNVモデルから60 ID50の感染性があると推定され、このロット全体で18.38 ID50が含まれていることになる。患者は8025単位の投与を受けたので0.16 ID50に相当する異常プリオンタンパクを投与されたと推定されている。 FHC4237: 21330人のプールから製造され、0.05 ID50に相当する異常プリオンタンパクを投与されたと推定されている。 一方、この患者は他に、39万単位の英国で採血された血漿由来の第Ⅷ因子製剤を投与されている。虫垂と扁桃の摘出検体の調査から、英国での献血者の中に未発症の感染者が1万人に1人存在していると推定されている。その推定に基づくと、2万人プールの原料血漿に2人の未発症の血漿が入っていることになり、ロット毎に感染価が異なるものの、平均すると第Ⅷ因子1単位あたり6X10^-5 ID50の異常プリオンタンパクが混入していたと推定される。その結果、患者に投与された総異常プリオンタンパク量は24 ID50に相当する達し、上記の2ロット以外のロットからの方が多くの異常プリオンタンパクに暴露されていたことになる。この患者は内視鏡、輸血等も受けていたが上記の計算から第Ⅷ因子製剤からの感染が疑われている。 問題点： 該当する製剤の製法が不明。また、linear dose-response modelを用いて個々のロットの感染リスクを加算することによってこの症例が、第Ⅷ因子製剤から感染したと推定しているが、化学物質と同様なことが異常プリオンタンパクの感染にも当てはまるのかについては不明である。さらに献血者1万人あたり1人未発症の感染者がいるという推定についても、献血後少なくとも10年が経つにもかかわらず発症者が問題となっているロットの1人のみということから考えても感染率を過大評価している可能性もある。また、現在も多くの感染者が潜伏期の状態にあるとした場合、10年から20年以上前に献血した血液中に感染性があるのか、という疑問が残る。しかし、該当する2ロットについては発症前の感染者の血漿が混入していたこと、及び他の文献等から当時の製造工程によっては最終製品にプリオンが混入する可能性があると考えられる。

資料番号			概要
A-2 血漿分画製剤における異常プリオンの不活化・除去法について			
②	Vox Sanguinis 2004;86(2):92-99	Distribution of a bovine spongiform encephalopathy-derived agent over ion-exchange chromatography used in the preparation of concentrates of fibrinogen and factor VIII	Scottish National Blood Transfusion Serviceにより第Ⅷ因子製剤「Liberate」について。S/D処理と陰イオン交換クロマトを用いた製造法により、異常プリオンタンパクの感染性はBSE由来の異常プリオン株BSE301Vで2.7Log（フィブリノゲンは2.9Log）除去される。なお、イムノクロマトを用いた方法では4.57Log除去されると報告。
③	Vox Sanguinis 2004;87 supply2:7-10	Removal of TSE agents from blood products	クリオプリシピテートの精製工程では、1log程度の異常プリオンタンパク除去効果があると報告。
④	Haemophilia 2002;8:53-75	Factor VIII and transmissible spongiform encephalopathy: the case for safety	第Ⅷ因子製剤「Liberate」について、異常プリオンの感染性は異常プリオンタンパク株263Kに対して6.8Log除去されると報告。
[illegible]	Vox Sanguinis [illegible]	Studies on the Removal of Abnormal Prion Protein by Processes Used in the Manufacture of Human Plasma Products	[illegible]デプスフィルター法にて[illegible]グロブリンよりも下流の分画から[illegible]証明されなかった。
⑤	Transfusion Medicine 1999;9:3-14	Assesment of the potential of plasma fractionation processes to remove causative agents of transmissible spongiform encephalopathy	各種クロマトグラフィーや各種フィルトレーション等の製造工程における異常プリオンタンパクの除去効果について検討。
[illegible]	[illegible]	The distribution of infectivity in blood components and plasma [illegible]	[illegible]
[illegible]	[illegible]	[illegible] chromatographic and ethanol [illegible]	[illegible]の分画法によるアルブミン製剤と免疫グロブリン製剤の[illegible]それぞれ[illegible]
[illegible]	[illegible] 2007;[illegible]:27-[illegible]	[illegible] removal [illegible] filtration under different experimental conditions	[illegible]15nmのサイズのプラノバでプリオンの除去が可能であったが、15nmのものが除去効率が[illegible]3Log前後の除去が可能であった。また、評価に用いるプリオン[illegible]であってプリオンの大きさを小さくするように調節することが必要である。

資料番号			概要
⑩	Biologicals 2006;34:227-231	CJD PrPsc removal by nanofiltration process: Application to a therapeutic immunoglobulin solution (Lymphoglobuline)	製造工程で人由来の赤血球や胎盤を使用するウマ抗人胸腺細胞免疫グロブリンにプリオン病に感染した人の脳乳剤を添加し、ナノフィルトレーションを行なったところ、1.6～3.3 Logのプリオン除去が可能であった。
A-3	白血球除去による異常プリオンの除去について		
⑪	Vox Sanguinis 2006;91:221-230	Creutzfeldt-Jakob disease and blood transfusion: results of the UK Transfusion Medicine Epidemiological Review study	白血球除去フィルターが導入された1999年以降、献血後にvCJDを発症した供血者から輸血を受けた27例の受血者については、今までのところ感染発症したとの報告はない。
⑫	Lancet 2004;364:529-531	Effectiveness of leucoreduction for removal of infectivity of transmissible spongiform encephalopathies from blood	白血球除去工程によって異常プリオンタンパクの感染性を60%減少させることができるが、すなわち血漿には感染性が40%残存する。(WBCは1X10^6/unit未満になっており赤血球製剤や血小板製剤の感染リスクはバッグに残存する血漿に依存している。文献②)
⑬	Lancet Neurology 2006;5:393-398	Predicting susceptibility and incubation time of human-to-human transmission of vCJD	ヒト型のプリオンタンパクを発現するトランスジェニック(Tg)マウスを作製し、BSE感染牛及びvCJD由来の脳乳剤をそれぞれ脳内接種により感染実験を行った。ヒト型TgにBSE由来異常プリオンタンパクは感染しなかったが、遺伝子型がMM(メチオニン・メチオニン)型やMV(メチオニン・バリン)型のTgはvCJD由来の異常プリオンタンパクに感受性を示し、VV型のTgに対しては他の遺伝子型よりも抵抗性を示したが感染が成立した。vCJDの感染効率はヒトからヒトの方が、牛からヒトよりも高い。
⑭	Blood 2008;112;4739-4745	Prion diseases are efficiently transmitted by blood transfusion in sheep	プリオン病が輸血で感染することをヒツジの系で詳細に解析した報告である。TSE発症前の状態を含めた異常プリオンに感染したヒツジの血液を輸血することによって、BSE由来のプリオン病では36%、scrapie由来のプリオン病では43%の輸血を受けたヒツジがプリオン病を発症した。これまでのマウスなどの小動物を用いた実験と異なり、ヒトの輸血に使用する量を投与できること及び長期間の観察が可能(マウスでは2年以下)な点がヒトに近い。
B. 英国、フランス、アメリカ、カナダにおける対応			
⑮	Transfusion 2009;49:797-812	From mad cows to sensible blood transfusion: the risk of prion transmission by labile blood components in the United Kingdom and in France	英国においてはBSE感染牛のピークから12年後にvCJD発生のピークを迎えている。現在、英国でのvCJD新規発症者数が減少しているが、今後70例(10～190例)くらい発症すると推定されている。一方、扁桃と虫垂における異常プリオン陽性率(3/12500例)から更に3000例発生すると推定されている。(現在、さらに10万検体を目標に追跡プロジェクトは進行している(0/45000、2008年))。この違いは感染者の93%が長い潜伏期の状態にいるとのことである。その結果からすると供血者の1/4000人が感染しており、血液や組織、医療機器を介して2次感染が起こる可能性がある。なお、虫垂から検出された2人の遺伝子型はVV型であった。 フランスでは1996～2007年に計23症例のvCJDが報告されている。この中には、供血後にvCJDを発症した3例が含まれている。これまでにこの3例の供血については、42人に投与され、うち16人が生存していることが判明している。
⑯	Brithish journal of haematology 2008;144:14-23	An update on the assessment and management of the risk of transmission of variant Creutzfeldt-Jakob disease by blood and plasma products	
⑰	Brithish journal of haematology 2005;132:13-24	Managing the risk of transmission of variant Creutzfeldt Jakob disease by blood products	

資料番号			概要
⑱	FDA(2009.6.18)	Questions and Answers on "Guidance for Industry: Revised Preventive Measures to Reduce the Possible Risk of Transmission of Creutzfeldt-Jakob Disease (vCJD) by Blood and Blood Products	アメリカにおいては、英国滞在歴通算3ヵ月以上、フランス滞在歴通算5年以上の者については献血制限を行う施策を続行する(2009.6.18現在)。
⑲	Health Canada	Donor Exclusion to Adress Theoretical Risk of Transmission of variant Creutzfeldt-Jakob Disease (vCJD) through the Blood Supply	カナダにおいては、1980～1996年に英国滞在歴通算3ヵ月以上、フランス滞在歴通算3ヵ月以上の者の献血制限を行っている。
⑳	Health Canada	Additional Donor Exclusion Measures to Address the Potential Risk of Transmission of variant Creutzfeldt-Jakob Disease (vCJD) through the Blood Supply	
㉑	オーストラリア		1980～1996年に英国滞在歴通算6ヶ月以上の者からの献血制限を行なっている

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

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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 [illegible] of the recent [illegible] of the spleen of a haemophiliac. This involves a patient [illegible] of potential vCJD infection routes (including [illegible] transfusions, repeated [illegible] of UK-sourced [illegible] including some units [illegible] a donor who later [illegible] vCJD, and several invasive biopsies) who was found [illegible] abnormal prion protein in a spleen sample.

2. If this finding is interpreted as an instance of [illegible] raises questions as to the operational meaning of [illegible] The discovery of abnormal protein in a single [illegible] result after exhaustive investigation of tissues [illegible] haemophilia patient who died of other causes [illegible] other neurological condition. All other tissues [illegible] presence of abnormal prion protein – fixed [illegible] vessel, appendix, spleen and lymph node and [illegible] occipital lobe, cerebellum, lymph node [illegible] were negative. This individual would not [illegible] vCJD prevalence tests conducted so far, and [illegible] spleen survey (depending on the size of [illegible] whether someone with this limited distribution [illegible] be infective - and if so, by what routes of [illegible]

3. For present purposes, however, these [illegible] simply assume that the abnormal prion protein [illegible] for asymptomatic vCJD infection: the [illegible] likelihood of the infection having come from [illegible] done in order to inform discussion by the CJD [illegible] to the implications of the finding, and in particular [illegible] warrants any change to the "at risk" status of [illegible]

4. The ideal would be to quantify these likelihoods [illegible] is not possible due to the multiple uncertainties [illegible] rehearsed. We do not know the prevalence [illegible] instance, some of the potential routes [illegible] are not, so the relativities change. The [illegible] component transmitting infection is uncertain [illegible] approach adopted by the Panel, it is [illegible] implicated plasma derivatives transmitting [illegible] However, they can be estimated using [illegible] assessment by independent consultants [illegible] used in drawing up Panel recommendations [illegible] been regarded as "precautionary", i.e. [illegible] infectivity likely to be present.

5. Given these unknowns, we make no attempt [illegible] though illustrative examples are provided. [illegible] limited task of determining whether different [illegible] contacts associated with the index patient can [illegible]

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.

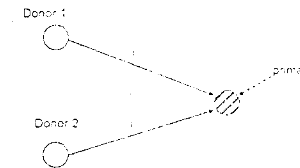
Method

7. The following analysis starts from the "reverse risk [illegible] by the Panel to assess the implied risks of donors to vCJD [illegible] infected (DH, 2005a; Bennett, Dobra and Gronlund, 20[illegible]) [illegible] with this much more complex incident. We start with [illegible] build up the analysis step-by-step. This is both to dem[illegible] conclusions are reached in this case, and to show how [illegible] used to handle other complex incidents that may arise.

Example 1

8. We therefore start with a simple incident as shown in Figure 1 [illegible] has received two single-unit Red Cell transfusions, one from [illegible] The recipient goes on to develop vCJD, and the timing of [illegible] not rule either of the donors out as the route of infection. [illegible] 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 chan[illegible] one of the donors were to be infected – i.e. the [illegible] definition, this lies between 0 and 1: if t = 1, transm[illegible] that case, and all else being equal[1], the patient's [illegible] have come from primary infection, or from either of [illegible] infected. So by implication, each donor would have a 1 [illegible] 3 [illegible]

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[1] "All else being equal" essentially means that there is no prior reason [illegible] recipient were particularly likely or unlikely to have been infected [illegible] risk" surgery, or conversely not having lived in the UK during years [illegible]

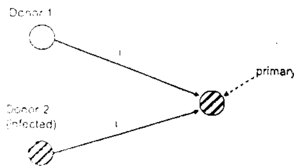
infective.[2] More generally, if there are n donors, the chance of each being infective would be 1/(n+1).

[illegible] 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

[illegible] 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 [illegible]

and P(prim) be the probability of the [illegible]

- For simplicity, suppose that the chance of the [illegible] than one route is negligible. Then (given that infe[illegible] P(D2) and P(prim) must add up to 1.

- Furthermore, the "balance" between the three [illegible] by t and p. Specifically:
  - P(D1) will be proportional to both [illegible] t (transmission probability)
  - P(D2) will only be proportional to [illegible]
  - and P(prim) will only be proportional [illegible]

12. Provided p is small (e.g. [illegible] or 1/10,000) [illegible] larger than either of the other two probabilities. [illegible] P(D2) = 1 and P(D1) and P(prim) are zero. [illegible] infection came from Donor 2. In practical terms, [illegible] Donor 2 means that D[illegible] need not be cons[illegible] CJD Incidents Panel [illegible]

Example 3

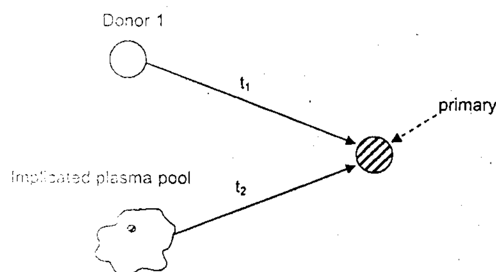
13. In the last two examples the two secondary ro[illegible] probability, t. But suppose now that there are [illegible] e.g. transfusion of blood components and rece[illegible] Figure 2 below shows a situation in which the [illegible] contrasting secondary routes:

   - a blood component transfusion, associated with a hi[illegible] probability (t_1) if the donor (D1) is infected but with [illegible] that this is the case, and
   - a plasma product [illegible] with a contributing donor [illegible] infected, but with a low transmission probability (t_2)

   As before, the three probabilities P(D1), P(D2) and P(prim) [illegible] 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:

    $P(D1) = 1/12$ $P(D2) = 10/12$ and $P(prim) = 1/12$

    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 [illegible] including nearly 400,000 units of Factor VIII, with no [illegible] "[illegible]ted" donors

To simplify the subsequent discussion, we consider the [illegible] from each of these routes in turn.

Transmission risks from the endoscopies

17. vCJD transmission risks from endoscopy have been examined [illegible] CDP [illegible] WG subgroup, informed by an outline risk assessment. It is [illegible] to appreciate that these procedures involve a very small [illegible] being passed down a very long, thin, channel. The possible [illegible] infection therefore differs from other surgical procedures. The [illegible] that [illegible] significant risk of onward transfer of infective material [illegible] would require the procedure to be invasive, as distinct from [illegible] involve the instrument sliding against the wall of the gut. On [illegible] the relative risk from endoscopic procedures *not* involving biopsy [illegible] negligible.

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

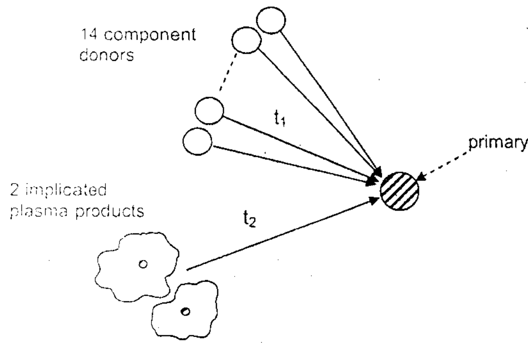
19. For endoscopy with re-usable heads, the best existing [illegible] the current surgical risk assessment as applied to procedures encountering [illegible] tissue. Depending on assumptions on the efficacy of decontamination [illegible] "standard" model suggests that indefinite re-use of a set of instruments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

20. As will be seen below, the chance of this particular patient having [illegible] infected by the primary route are very small (in all scenarios) as compared [illegible] of infection through a blood-borne route. On the above [illegible] the same applies to the endoscopic route. For simplicity, this route will [illegible] regarded in the following calculations. It should be noted that [illegible] if [illegible] of transmission via endoscopy were much greater than [illegible] the only effect on subsequent calculations would be to reduce [illegible] 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 10,000 for the prevalen[illegible], p. The [illegible] infection having come fr[illegible] implicated plasma pr[illegible] 14 component (Red Cell) donors, and from the pri[illegible] that in all scenarios, the [illegible] illustrative figures, using assumptions subject to [illegible] they do suggest that the infection is much more lik[illegible] plasma products, with the implied risk [illegible] clearly below 1%.

Table 1: Relative probabilities of potential infection [illegible] implicated plasma" products

Prevalence, p	[illegible]
Transmission probability, t1	[illegible]
Probability implicated plasm[illegible]	[illegible]
Probability of each of the 14 [illegible]	[illegible]
Probability primary	[illegible]

Note: these are illustrative [illegible] probabilities) indicate an upper [illegible]

Implicated and "Non-implicated" p[illegible]

25. Although the above analysis provides some rob[illegible] infection routes considered so far, the calculations ign[illegible] chance of the infection [illegible] the [illegible] – i.e. those manufactured from plasma pools not [illegible] contributing donor. The [illegible] is that [illegible] (of the order of 20,00[illegible] there is [illegible] them did, in fact, contain [illegible] even if [illegible] Crudely, if the prevalen[illegible] contain about 2 infected [illegible]

26. This argument does no[illegible] non-implicated pools. [illegible] donor (and nothing is [illegible] have the same probab[illegible] 10,000 and typical poo[illegible] implicated" pool to [illegible] contain 3. Neverthele[illegible] that unless the prevalen[illegible] here, there is only a m[illegible] and non-implicated pla[illegible] considering recipients [illegible]

[3] More strictly, the expected number of infected donations in each [illegible] distribution. However, the distri[illegible] not essential to the argu[illegible] receiving high volumes of product [illegible] from many different [illegible] fluctuations will tend to even out

applying additional measures to those with known exposure to implicated batches.

27. This specific haemophilia patient had received such large quantities of Factor VIII – almost 400,000 units, the majority since 1980)] - that on these calculations, the cumulative risk from the "non-implicated" batches may well have exceeded that from the smaller number of "implicated" ones. This can be illustrated by considering the expected number of ID_{50} received via each route. This is illustrated in the second part of Annex A. In summary:

- If the two "implicated" pools contained 3 infected donations, this route would have exposed the patient to a total dose of 0.6 ID_{50}.
- If the other "non-implicated" pools each contained 2 infected donations, this route would have exposed the patient to an expected total of 24 ID_{50}.

28. Simple application of the linear dose-response model would then suggest that whereas Factor VIII from the two "implicated" pools would have contained a dose liable to transmit infection with a probability of 0.3, the large number of units sourced from "non-implicated" pools would have contained more than enough infectivity to transmit. Crudely, this suggests that the "non-implicated" pools represent the more probable source of infection, by a factor of just over 3.[4]

29. This last calculation is reflected in Table 2 below, for prevalence scenarios of both 1 in 10,000 and 1 in 4,000. However, we stress that this is very simplistic. It rests on accepting the linear model uncritically, and assuming that doses received on successive occasions can simply be added together in calculating an overall risk of infection. Nevertheless, the comparison between "implicated" and "non-implicated" routes is instructive, in showing how the sheer number of exposures may come to dominate the presence of a known infection.

Table 2: Relative probabilities of potential infection routes (including "non implicated plasma" products)

Prevalence, p	1 in 4,000		1 in 10,000	
Transmission probability, t1	0.5	1	0.5	1
Probability implicated plasma products	38%	38%	24%	24%
Probability of each of the 14 component donors	<0.03%	<0.03%	<0.02%	<0.02%
Probability primary	<0.03%	<0.03%	<0.02%	<0.02%
Probability non-implicated plasma products	61%	61%	76%	76%

Note: these are illustrative calculations only. All figures are rounded to the nearest %, or (for small probabilities) indicate an upper bound.

4 Note that the differential between *infectious doses* is much greater, but the practical effect is limited by infection being regarded as certain once the dose reaches 2 ID_{50}. As seen in following paragraphs, the risk differential between routes is therefore more pronounced in lower-infectivity scenarios.

30. As can be seen, the previous conclusion about the low implied [illegible] of the 14 component (red cell) donors still applies, with even greater [illegible]. However, these results also highlight something of a paradox. Combined [illegible] infectivity scenario taken from the DNV assessment, the pool size [illegible] prevalence calculations suggest that many recipients of plasma products [illegible] very high infectious doses, *whether or not* they had received any [illegible] with known linkage to an infected donor. This opens the question of why no clinical vCJD cases have been seen in the population of haemophilia [illegible] blood disorder patients designated as "at risk" because of their exposure [illegible] sourced blood products.[5] It might therefore be argued that the infectivity [illegible] assumptions applied to plasma products are overly pessimistic.

31. Although this question is impossible to answer definitely, and [illegible] issues beyond the scope of this paper, it is appropriate [illegible] conclusions we have already suggested about relative [illegible] be overturned were we to assume lower levels of infectivity [illegible]. The DNV report itself suggests two possible methods [illegible] infectivity present in each plasma derivative, using different [illegible] effect of the various manufacturing steps. In line with the [illegible] precautionary approach adopted by CJD Incidents Panel, [illegible] use figures based on the more pessimistic of these. The [illegible] alternative suggested by DNV (using the "highest single [illegible]" in the manufacturing process) leads to an infectivity estimate for [illegible] is lower by a factor of 4. However, it should also be noted [illegible] carried out elsewhere take the clearance factors achieved [illegible] at least partly additive, which would lead to much smaller [illegible].

32. In fact, reducing the assumed infectivity *increases* the relative [illegible] via "non-implicated" as compared to "implicated" plasma. [illegible] the presumed infectivity in all the Factor VIII received was [illegible] 100 (2 logs). Modifying the calculations in paragraph 27, [illegible] have received an expected:

- 0.006 ID_{50} from the two "implicated" pools (representing a [illegible] of 0.003)
- 0.24 ID_{50} from all the other "non-implicated" pools [illegible] infection risk of 0.12).

33. Albeit with the same caveats as before about using the [illegible] the cumulative risks from successive doses, this suggests [illegible] outweigh the former by a factor of 40. Table 3 shows [illegible] for this patient would change under this revised infectivity [illegible].

5 *Possible* explanations include the following: [illegible] prevalence of infection [illegible] lower than in the scenarios considered [illegible] much more infectivity [illegible] processing of plasma products than suggested by the DNV analysis, [illegible] dose-response effect and most recipients fall below this. Genotype [illegible] providing resistance to infection or extending the time to clinical disease, [illegible] substantial proportion of this group to be MM homozygotes - the most [illegible]

[illegible] conclusions still hold, in particular regarding the small implied risk to each of the 14 red cell donors.

Table 5: Relative probabilities of potential infection routes (including "non implicated plasma" products and using lower infectivity estimates for plasma products)

Prevalence, p	1 in 4,000		1 in 10,000	
Transmission probability, t1	0.5	1	0.5	1
Probability implicated plasma products	2%	2%	3%	3%
Probability of each of the 14 component donors	<0.05%	<0.09%	<0.05%	<0.09%
Probability primary	<0.09%	<0.09%	<0.09%	<0.09%
Probability non-implicated plasma products	97%	97%	97%	96%

Note: [illegible] illustrative calculations only. All figures are rounded to the nearest %, or (for small probabilities) indicate an upper bound.

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Annex A: Application of DNV Risk Calculation [illegible]

(a) Implicated Donations

Key points: FHB4547

- There was one implicated [illegible] donations (pool size supplied [illegible] Frank Hill [illegible])
- Factor VIII is derived from [illegible], which [illegible] ID_{50}s / donation of infected [illegible]
- 70.45kg of cryoprecipitate [illegible] in the FHB4547 batch.
- This implies that (21.5[illegible]kg [illegible] batch (18.38 ID_{50}s)
- 1,844 vials each of 500 [illegible] estimate of 0.00997 ID_{50}s [illegible]

Professor Frank Hill's report indicates that the index case [illegible] batch, giving an estimated [illegible] from the implicated donation.

Key points: FHC4237

- There was one implicated [illegible] donations (pool size again [illegible] Professor Frank [illegible])
- Factor VIII is derived from [illegible], which [illegible] ID_{50} / donation of whole [illegible]
- 67.6kg of cryoprecipitate [illegible] start [illegible] FHC4237 batch
- This implies that the full [illegible]
- 5,074 vials each of 250 [illegible] 0.0118 ID_{50} per vial or [illegible]

Professor Frank Hill's [illegible] batch, giving an estimated dose [illegible]

Conclusion

In total, these calculations suggest [illegible] ID_{50} from the "implicated" [illegible] translates into a transmission probability of [illegible] transmission probability of [illegible] 10.4%.

(b) Non-implicated Donations

In addition to the implicated donations, we have also to consider the possibility of other donors contributing to a pool being infective. With pool sizes of the order of 20,000 donations, each pool will be likely to contain contributions from one or more infected donors by chance, unless p is very small. For implicated pools, these will be *in addition to* the "known" implicated donor.

With a prevalence of 1 in 10,000, one might therefore expect the two implicated pools to contain two *further* infected donations, taking the total from 1 to 3 per pool.

This would make the infective dose received via the implicated units three times that calculated above, i.e. a total of roughly 0.6 ID_{50}, yielding a transmission probability of 0.3.

This patient also received approximately 391,000 iu of UK-sourced Factor VIII plasma treatment *not* known to be associated with any infected donor. In round figures, this can be visualised in terms of 20 exposures to pools of 20,000 donors, each typically containing 2 donations from infected donors. The exact infective dose passed on to the patient will vary from batch to batch. However, the two examples given in part (a) suggest an eventual dose of 2-5 x 10^{-5} ID_{50} per unit, per infected donor. For illustration, therefore, suppose that each unit exposed the recipient to 6 x 10^{-5} ID_{50}, 400,000 such units would therefore have exposed the recipient to 24 ID_{50}.

ORIGINAL PAPER

Distribution of a bovine spongiform [illegible] -derive[illegible] agent over ion-exchange chromato[illegible] preparation of concentrates of [illegible] VIII

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Background and Objectives [illegible] Jakob disease [illegible] via treatment [illegible] fore, in order to determine [illegible] the preparation of factor VIII [illegible] encephalopathy (BSE)-derived agent [illegible] used to prepare the Scottish National [illegible] VIII concentrate (Liberate).

Materials and Methods Murine-passaged BSE [illegible] somal fraction prepared from infected brain [illegible] VIII of intermediate purity. The 'spiked' [illegible] detergent treatment and then to anion-exchange [illegible] with Toyo[illegible] DEAE-650M. All fractions were tested for 301V [illegible] murine [illegible] including the procedures used to clean the ion [illegible] for use.

Results BSE 301V infectivity was [illegible] target fractio[illegible] by 2·7 log_{10} in the factor VIII fraction. [illegible] 301V infecti[illegible] remained bound to the ion-exchange [illegible] factor VIII. A quantity of infectivity was subsequently [illegible] ion-excha[illegible] media with 2 M NaCl. No further BSE [illegible] column cl[illegible] after treatment with 0·1 M NaOH or [illegible]

Conclusions Results using a BSE-[illegible] infectivity [illegible] be substantially removed by the ion-exchange [illegible] preparati[illegible] fibrinogen and factor VIII concentrates [illegible] bound to the ion-exchange matrix [illegible] be eliminated by the proce[illegible] each use.

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Key words: Creutzfeldt-Jakob [illegible]

Introduction

Variant Creutzfeldt–Jakob disease (vCJD) is an incurable, fatal, neurodegenerative disorder of transmissible spongiform encephalopathy [illegible] been [illegible] firmed or [illegible] the condition [illegible] first reported [illegible] were resident [illegible] the UK at [illegible] is caused by [illegible] TSE agent responsible [illegible] spongiform encephalopathy (BSE) is carried [illegible] dietary exp[illegible] being the most [illegible] transmission [1] [illegible] originated in the UK [illegible] detected in ca[illegible] in 25 different countries [illegible] of all cases [illegible]

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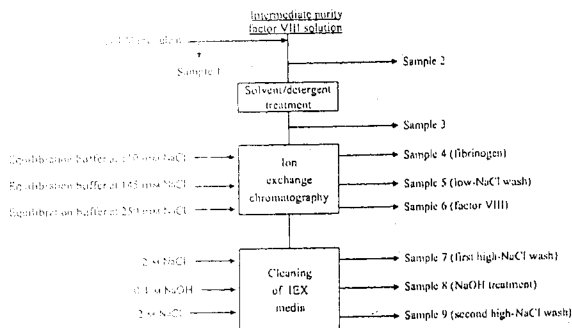

Fig. 1 Flow diagram of the processes over which partitioning of bovine spongiform encephalopathy (BSE) 301V infectivity was measured. IEX, ion-exchange chromatography.

of equilibration buffer, with the breakthrough (unadsorbed) fraction (139·8 ml) being collected (fibrinogen fraction). Forty-one millilitres of equilibration buffer, containing 145 mM NaCl, was then applied and the resultant wash fraction collected (low-NaCl wash). This was followed by 26 ml of equilibration buffer containing 250 mM NaCl, at a flow-rate of 48 ml/h, to elute factor VIII (factor VIII fraction).

Cleaning of the ion-exchange gel

Following collection of the factor VIII eluate, the chromatography bed was cleaned *in situ* by washing with 2 M NaCl, followed by 0·1 M NaOH and then again with 2 M NaCl. First, 25 ml of 2 M NaCl was applied to the column and the eluate (15·2 ml) was collected from the beginning of the 'salt front' (first high-NaCl wash). Subsequently, 0·1 M NaOH (70 ml) was applied to the column and an eluate (39 ml) was collected when the pH increased from 6·3 to > 12 (NaOH wash). When the application of 0·1 M NaOH was complete, the column was allowed to soak in NaOH for 1 h and then subjected to a second wash with 2 M NaCl (42 ml). An eluate volume of 8·1 ml was collected to capture the protein-containing fraction observed at this stage (second high-NaCl wash).

Determination of protein elution during the ion-exchange process

Throughout the ion-exchange procedure, the output from the column was monitored continuously by inline measurement of the solution optical density at a wavelength of 280 nm (OD_{280}) to detect total protein being eluted (Fig. 2).

Scale-down of the ion-exchange process

The small-scale ion-exchange procedure used in this study was designed to give yields and purification for factor VIII

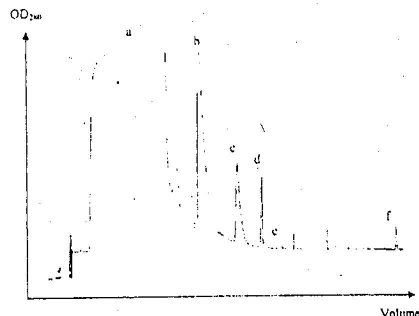

Fig. 2 Optical density of fractions eluted during ion-exchange chromatography of intermediate-purity factor VIII to which the bovine spongiform encephalopathy (BSE) 301V microsomal inoculum had been added. (a) Fibrinogen fraction (110 mM NaCl); (b) low-NaCl wash (145 mM NaCl); (c) factor VIII fraction (250 mM NaCl); (d) first high-NaCl wash (2 M NaCl); (e) NaOH wash (0·1 M NaOH); (f) second high-NaCl wash (2 M NaCl).

and fibrinogen equivalent to the full-scale process. Although the degree of scale-down was ≈ 1300-fold, all materials and surfaces were the same as in routine manufacture, except that chromatography eluates were collected into polypropylene containers rather than stainless steel vessels. The OD_{280} profile obtained in the presence of added 301V (Fig. 2) was the same as that obtained in the absence of 301V, both in the small-scale model and in the routine full-scale chromatography process, demonstrating the accuracy of down-scaling achieved.

Determination of BSE 301V infectivity

The BSE 301V infectivity of samples from the ion-exchange process was determined by bioassay. Samples for assay were diluted in saline and injected intracerebrally (20 μl) into

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Table 2 Distribution of bovine spongiform encephalopathy (BSE) 301V infectivity [illegible] factor VIII by ion-exchange chromatography

Stage/fraction	BSE titre (ID_{50}/ml; log_{10})	Volume of fraction (ml)	[illegible]	[illegible]	[illegible]
1. Microsomal inoculum	7·3[a]	[illegible]			
Factor VIII process					
2. Factor VIII solution (spiked)	5·7	[illegible]			
3. Factor VIII solution after S/D[b]	6·6	[illegible]		[illegible]	
4. Fibrinogen fraction (120 mM NaCl)	< 3·8	[illegible]		[illegible]	[illegible]
5. Low-NaCl wash (145 mM NaCl)	< 3·4[d]	[illegible]		[illegible]	[illegible]
6. Factor VIII fraction (250 mM NaCl)	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
Column cleaning					
7. First high-NaCl wash (2 M NaCl)	6·4[d]	[illegible]		[illegible]	[illegible]
8. NaOH wash (0·1 M NaOH)	< 3·2	[illegible]	[illegible]	[illegible]	[illegible]
9. Second high-NaCl wash (2 M NaCl)	< 3·2	[illegible]	[illegible]	[illegible]	[illegible]

[a]Transmissible spongiform encephalopathy (TSE) titre [illegible] [24].
[b]S/D, after treatment with solvent and detergent.
[c]Maximum value on the assumption that 100% of animals would have been positive [illegible]
[d]Approximate TSE titre, estimated from bioassay at one dilution using the dose-response [illegible]
ID_{50}, infectious doses 50%.

consistent with the original level of infectivity, suggesting that aggregates may have formed during the frozen storage of the microsomal fraction and that full dispersion was only achieved after the microsomal fraction had been added to the solution of intermediate-purity factor VIII. Although there was a small apparent increase in 301V titre following solvent/detergent treatment (Table 2), this was well within the margin of error for TSE bioassay titrations. [illegible] a small increase in TSE titre is often detected after [illegible] detergent treatment or other disaggregating treatment [illegible] cation) and is probably a result of disaggregation, but may also occur as an effect of the efficiency of titration [illegible].

The three fractions recovered from the ion-exchange process, including the factor VIII fraction, all contained 301V infectivity. However, the quantity of infectivity present in each of these fractions was much less than that of the starting material. From these data it was calculated that with respect to the feedstock to ion-exchange chromatography, 301V infectivity was reduced by 2·9 log_{10} in the fibrinogen fraction and by 2·7 log_{10} in the factor VIII fraction (Table [illegible]). It was also estimated that less than 0·4% of the 301V infectivity present in the feed to the ion-exchange process (sample 3) was recovered in the fractions collected up to and including the factor VIII fraction (Table 2), indicating that 99·[illegible] of the added infectivity remained bound to the ion-exchange matrix following the recovery of factor VIII.

In the procedure used to clean the ion-exchange gel between uses, we found that a significant degree of infectivity desorbed into the first 2 M NaCl wash (Table 2). Subsequently,

Discussion

agent. We used a microsomal fraction for this purpose, for two reasons: first, by removing whole cells and large fragments, the method of preparation was similar to the separation of plasma from whole blood; and, second, to permit comparison of the results from this study with those from our earlier experiments with the scrapie agent in which a microsomal fraction was also used [16,24]. No specific measurements were performed to characterize the microsomal fraction, other than to titrate it for TSE infectivity. However, no significant TSE reduction has been observed over leucofiltration, using either endogenously infected murine plasma [41] or blood spiked with the microsomal fraction [42], indicating that, with respect to leucofiltration, the microsomal fraction contains PrP^{Sc} of a comparable state to that derived from an endogenous source. Nevertheless, the extent to which 301V infectivity from the micosomal fraction represents the vCJD agent as it would exist naturally at the intermediate stage of the factor VIII manufacturing process, has still to be established. Finally, our measurements on the procedure used to clean the ion-exchange matrix, and our inability to achieve an exact mass balance, were limited by the sensitivity of the murine bioassay (Table 2). This was constrained by dilution of the samples to make them suitable for intracerebral inoculation, the small volume of sample tested and the number of animals employed, which was minimized for ethical reasons.

Conclusions

This experiment has resulted in a number of important observations. First we have confirmed that ion-exchange chromatography can substantially remove a BSE-derived agent from preparations of fibrinogen and factor VIII concentrate. Second, most of the added TSE agent remained bound to the ion-exchange matrix after elution of factor VIII. Third, the cleaning procedure used to sanitize the ion-exchange matrix between uses was effective in eliminating a significant proportion, and possibly all, of the BSE-derived agent that remained bound after the elution of factor VIII. Finally, our results were similar to those obtained previously using hamster-adapted scrapie, suggesting that scrapie 263K may be a suitable TSE model for using to estimate the partitioning behaviour of the vCJD agent over ion-exchange chromatography.

Acknowledgements

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Removal of TSE agents from blood products

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Introduction

Transmissible Spongiform Encephalopathies (TSEs) are fatal neuro-degenerative disorders. Creutzfeldt-Jakob disease (CJD) in humans is divided into classical CJD (cCJD), of which there are a number of forms (sporadic, familial, Gerstmann-Sträussler-Scheinker (GSS) syndrome), and variant CJD (vCJD), the latter probably transmitted by food contaminated with bovine spongiform encephalopathy (BSE).

cCJD has been transmitted by medical procedures in which tissues with a high level of infectivity were involved [1] but transmission by blood products has not been observed [2] possibly because infectivity in blood is very low. By contrast, vCJD has probably been transmitted by transfusion of whole blood [3] consistent with experimental transmissions of BSE between sheep [4].

The prevalence of cCJD is 0·5–1·0 per million inhabitants per annum world-wide [5]. About 150 cases of vCJD have been recorded, but the subclinical prevalence of infection in the human population is not known. BSE has been discovered in over 20 countries and it is conceivable that large numbers of people have been exposed to infection. Without a suitable diagnostic test, the extent to which CJD agents may be present in blood donations is not known. It is therefore important to establish the extent to which TSE agents can be eliminated during the preparation of blood products.

TSE diseases are associated with conversion of prion protein (PrP) to a pathogenic conformation (PrPSc) that accumulates in the brain causing degeneration. TSE agents have been found to be highly resistant to physical and chemical treatments and methods for their inactivation [6] are too severe to be applied to blood products. Attention has therefore concentrated on removal using separations technologies. PrPSc has a number of properties which could be exploited to separate it from other biological substances; including a low solubility in aqueous solution, the ready formation of aggregates and a tendency to adhere to surfaces [7].

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Experimental approaches

Studies on the removal of TSE [illegible] infectivity in blood is very [illegible] agent as it exists naturally [illegible] experimental approaches [illegible] sary to appreciate their [illegible]

Process scale-down

Experiments with infective material [illegible] containment facilities as TSE agents represent a bio-hazard. This, together with the difficulty of [illegible] suitable infected tissue means that process studies are [illegible] undertaken at small volume, typically [illegible] whereas manufacturing processes operate at [illegible] For results to be meaningful it is necessary to simulate the manufacturing operation [illegible]

Form of TSE agent

Two basic forms of TSE material have been [illegible] obtained from experimentally infected animals [illegible] preparations derived from [illegible] such as brain homogenate (BH) [illegible] caveolae-like domains (CLDs) [illegible] [10]. Studies with infected [illegible] genous' whilst those using [illegible] as 'exogenous'. In endogenous [illegible] infectivity means that only a small degree [illegible] beginning of multistep processes [illegible] titre of infectivity available [illegible] enables greater capacities [illegible] and more steps considered. However, [illegible] over the extent to which materials [illegible] represent TSE agents present naturally [illegible]

Strain of TSE agent

Partitioning studies have been undertaken [illegible] TSE strains. Endogenous studies have [illegible] with murine-adapted GSS, Fukuoka-1 strain [illegible] hamster-adapted scrapie, strain 263K (R. G. Rohwer, unpublished) and murine-adapted BSE, strain 301 V (H. E. [illegible] unpublished). Exogenous

experiments have employed high titre preparations infected with hamster-adapted scrapie (strains 263K [9], Sc237 [10] and ME7 [11]), murine-adapted BSE, 301 V [12] and three strains of human CJD, vCJD, sporadic CJD (sCJD) and GSS [13].

Determination of TSE agents

Two approaches have been used to determine the degree of removal of TSE agents: measurement of infectivity by rodent bioassay [6,12,14,15] and immuno-chemical determination of PrPSc using either Western blotting [9,16] or conformation-dependent immunoassay (CDI) [10]. Immunoassays are performed after PrP has been removed by digestion with proteinase K (PK), PrPSc being resistant. Immunoassays are therefore dependent on the effectiveness of PK-digestion and the assumption that PrPSc is the infective agent, or that it partitions precisely with infectivity.

Studies on individual process steps

Leucocyte-filtration

Universal leucocyte-depleting-filtration of blood components was introduced as a precaution against vCJD transmission [17] following a report that B-lymphocytes were crucial to the pathogenesis of TSE disease [18], despite earlier findings [19]. In a small-scale study Brown *et al.* [14] filtered fresh plasma from symptomatic mice infected with GSS (Fukuoka-1 strain) using a white cell-reduction filter (Pall PLF1); no significant reduction in TSE infectivity was observed. Filtration has been studied at full-scale using a whole blood leucocyte-depleting filter (Pall WBF2) to filter 450 ml of blood from hamsters infected with scrapie-263K. Although infectivity was reduced by 42% (R. G. Rohwer, unpublished), this was within the error of the bioassay. Scrapie-263K was also employed in an exogenous experiment in which human blood spiked with MF was filtered using four different whole blood filters. Abnormal fragmentation of red cells occurred suggesting interference by the MF spike; nevertheless, no significant removal of PrPSc was observed over any of the filters [20]. Consequently, the ability of leucocyte-depleting filters to remove TSE agents from blood components has still to be established.

Protein precipitation

Separation of proteins according to differences in solubility is central to the manufacture of many plasma products. TSE partitioning has been studied over cryoprecipitation and a number of cold-ethanol precipitation steps (Table 1). Fraction III and Fraction IV, which are discarded from immunoglobulin and from albumin, respectively, gave a high degree of TSE removal. Separation is only achieved when the precipitate phase is removed from the solution phase. In routine manufacture, centrifuge supernatants are clarified by depth filtration to ensure that the resultant solutions are of uniform quality. Such filtration procedures are therefore an important adjunct to precipitation processes.

Depth filtration

In immunoglobulin manufacture, the supernatant remaining after removal of Fraction III (Supernatant III) and the solution obtained when Fraction II precipitate is re-dissolved are both subjected to depth filtration. Similarly in the preparation of albumin, both Supernatant IV and the solution obtained when Fraction V is re-dissolved are both treated by depth filtration. In these applications, added infectivity or PrPSc was removed to the limit of detection by Seitz filters, whereas filters from other manufacturers have given variable results (Table 2). PrPSc was not removed from Supernatant I by Seitz filtration [10], suggesting that the much broader spectrum of proteins present at this earlier stage of fractionation saturated the relevant binding sites on the filter. There are many types and grade of depth filter available and more comprehensive data are required to better define those suitable for removal of TSE agents.

Table 1 Removal of TSE agents by precipitation, with each process studied individually

	Foster	Lee	Lee	Stenland	Vey	Reichl
Ref	[9]	[15,16]	[15]	[13]	[10]	[12]
TSE agent	263K	263K	263K	vCJD	Sc237	301 V
spike	MF	BH	BH	BH	BH/MF/CLD/PrPSc	MF
assay	W blot	W blot	bioassay	W blot	CDI	bioassay
log_{10} Redn						
cryoppte	1·2	1·0	1·0	0·9	0·3/0·2/0·4/2·4	
fraction I		1·1			0·9/0·9/0·7/3·1	
fraction II + III	1·3	≥ 4·7	6·0		3·6/3·1/3·1/4·0	
fraction (I) + III	≥ 3·7	≥ 4·3	5·3			2·1
fraction IV_1/IV_4		≥ 4·2/≥ 4·1	3·7/4·6			
fraction IV	≥ 3·0				3·2/3·4/3·2/2·2	

Studies on process steps in sequence

As well as characterizing process steps [illegible] important to examine steps operated in s[illegible] mine if removal by successive steps is ad[illegible] precipitation steps in plasma fractionation [illegible] in endogenous [8,14, R. G. Rohwer, unpublis[illegible] unpublished] and in exogenous [8,25] [illegible] results (Table 3) demonstrate a progressive [illegible] the TSE agent over successive steps, indicating [illegible] precipitation processes can complement o[illegible] When precipitation was combined with depth [illegible] where two different filtration procedures [illegible] [12,22], the overall degree of TSE removal [illegible] the first step but was less than the sum obt[illegible] individual steps. These findings indicate that care [illegible] taken in interpreting data obtained only from individual steps.

Conclusions

There is a body of data suggesting that pr[illegible] plasma products are manufactured are cap[illegible] TSE agents. Nevertheless, there is uncert[illegible] relevance of the spiking materials used [illegible] experiments and the range of steps studie[illegible] experiments has been restricted. Methods [illegible] limited in sensitivity, and possibly in spec[illegible] studies are required, with advances in det[illegible] determine the safety of plasma products.

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REVIEW ARTICLE

Factor VIII and transmissible spongiform encephalopathy: the case for safety

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Summary. Haemophilia A is the most common inherited bleeding disorder, caused by a deficiency in coagulation factor VIII (FVIII). Current treatment of haemophilia A is based on repeated infusions of plasma-derived FVIII concentrate or of recombinant FVIII, which may be exposed to plasma-derived material of human or animal origin used in its tissue culture production process. We review epidemiological and experimental studies relevant to blood infectivity in the transmissible spongiform encephalopathies (TSEs, or 'prion' diseases), and evaluate the hypothetical risk of TSE transmission through treatment with plasma-derived or recombinant FVIII.

Keywords: blood, factor VIII, prion disease, safety, transmissible spongiform encephalopathy, variant Creutzfeldt–Jakob disease

Haemophilia and replacement therapy

According to a survey of the World Federation of Haemophilia, approximately 400 000 individuals worldwide are affected with hereditary bleeding disorders that require lifetime therapeutic care. Haemophilia A is the most common bleeding disorder, which affects 1 : 5000 males and is caused by a deficiency or functional defects in coagulation factor VIII (FVIII) [1]. Haemophilia B or Christmas disease affects 1 : 30 000 males [2] and is caused by a hereditary defect in coagulation factor IX (FIX). Both conditions are X-linked recessive disorders caused by mutations in the corresponding genes, and are passed to the next generation through the female line. von Willebrand disease is a rare haemorragic condition, inherited in autosomal dominant fashion, caused by a deficiency or defect of von Willebrand factor (vWF), which leads to a secondary deficiency of FVIII [3].

FVIII is an essential component of the intrinsic pathway of the blood coagulation cascade. It serves as a cofactor for a serine protease factor IXa (FIXa), which, in its membrane-bound complex (Xase), activates factor X [4,5]. Activated factor X (FXa) then participates in the conversion of a zymogen prothrombin into thrombin, a key enzyme of the coagulation cascade. Subsequently, thrombin cleaves fibrinogen to fibrin and activates FXIII, which leads to formation of a stable clot. Immediately after release into circulation, FVIII binds to vWF to form a tight noncovalent complex. Association with vWF is required for maintaining the normal FVIII level in circulation and for preventing the interaction of FVIII with other components of the intrinsic Xase complex. In addition, vWF protects FVIII from inactivation by activated protein C, and activated FIX and FX. Upon activation of the FVIII/vWF complex by thrombin, FVIII is rapidly released from the complex with vWF [4,6].

While initiation of blood coagulation is ascribed to the extrinsic, tissue factor-dependent pathway in which small amounts of activated factors IX and X are generated, the intrinsic pathway catalyses activation of factor X approximately 50-fold more

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efficiently, dramatically amplifying the coagulation events triggered by the tissue factor-dependent pathway [7]. The requirement of a powerful amplification of the coagulation burst via the FVIII-dependent intrinsic pathway for maintaining normal haemostasis explains why the absence of FVIII disturbs the coagulation process and results in haemophilia A.

Based on the residual activity of FVIII in plasma, haemophilia A is categorized as severe (< 1 IU dL^{-1} of normal activity), moderate (1–5 IU dL^{-1} of activity) and mild (5–30 IU dL^{-1}). Clinically, the severe form of the disease is characterized by spontaneous recurrent painful bleedings into joints, muscles and soft tissues, and may result in a chronic and debilitating arthropathy. Haemophilic pseudotumours may occur in bones as a result of repeated subperiosteal haemorrhages with bony destruction and new bone formation. More serious complications and death can result from bleedings into the intracranial and retroperitonial space.

Current treatment of haemophilia A is based on correcting functional FVIII deficiency by intravenous infusions of plasma-derived, affinity-purified and, more recently, recombinant FVIII products [8]. Plasma-derived concentrates of FVIII became available for the treatment of haemophilia A in the early 1960s and provided a dramatic improvement in the life expectancy of haemophilic patients [9]. Due to a relatively short half-life of FVIII in circulation (12–14 h) [10], treatment of haemophilia A requires repeated (up to three per week) infusions of expensive FVIII products and in cases of severe disease, the cost of treatment may be as high as US$100 000 per year. The major disadvantage of plasma-derived FVIII therapy was the risk of transmission of blood-borne viruses, such as hepatitis B and C and human immunodeficiency virus [9, 11]. Recombinant gene technologies offer new therapeutic products that are considered safer in certain aspects than plasma-derived concentrates [12–14]. The safety of plasma-derived concentrates has greatly improved in the last decade because of careful donor selection, screening of donations for infectious viruses, and enhanced efficacy of specific antiviral steps in the manufacturing process [15]. Concerns remain about the transmission of thermo-resistant nonlipid-enveloped viruses, such as parvovirus [16], which may be addressed, in part, by introduction of testing using polymerase chain reaction, and the hypothetical risk of transmission associated with variant Creutzfeldt–Jakob disease (vCJD) [13].