Ａ　研究報告概要

○　一覧表（感染症種類毎）
○　感染症毎の主要研究報告概要
○　研究報告写

研究報告のまとめ方について

１　平成１８年９月１日以降に報告された感染症定期報告に含まれる研究報告（論文等）について、重複している分を除いた報告概要一覧表を作成した。

２　一覧表においては、前回の運営委員会での報告したもの以降の研究報告について、一覧表の後に当該感染症の主要研究報告の内容を添付した。

感染症定期報告の報告状況（平成18年9月1日～平成18年11月30日）

血対ID	受理日	感染症(PT)	出典	概要	
60121	2006/09/29	A型肝炎	Epidemiol Infect 2006; 134: 87-93	1998年から1999年の韓国の血友病患者におけるHAV感染と血液凝固因子との因果関係を調べるため、比較対照試験と分子学的HAV検出を行った。疫学的調査およびHAV RNA配列検査から、凝固因子VIIIの1ロットがHAV感染に関与していたことが明らかになった。	
60121	2006/09/29	B型肝炎	Transfusion 2006; 46: 1256-1258	ヨーロッパでのB型肝炎発生率は北西部が低く(1%以下)、南部が高い(5-15%)。北西部では抗HBcスクリーニングが導入され、南部ではHBV-NATが導入される傾向がある。B型肝炎ウイルス陽性となった供血者と血液の管理に関して、ヨーロッパではHBV DNA、HBc抗体陽性でHBs抗体レベルが100IU/L以上の場合は供血を続けることができるというリエントリー・アルゴリズムが検討されている。	1
60121	2006/09/29	B型肝炎 C型肝炎	日本輸血学会雑誌 52(2): 197 第54回日本輸血学会総会 2006年6月 9-11日	輸血用血液製剤のHBV、HCV、HIVについて、2000年2月から2004年1月までの4年間の遡及調査を行った。調査は、主に複数回献血者において感染症マーカーが陽転した場合に前回の血液サンプルを個別NATで精査するもので、HBVについては50プールNAT, HBsAg, HBc Abのいずれかの陽転例約16000人について前回の保管検体を調べた。副作用報告による感染例を合せると、日本では輸血によるHBV感染が1年に約19例、HCV感染は4年に1例、HIV感染は2年に1例起こるものと推定された。	2
60121	2006/09/29	B型肝炎 C型肝炎	日本輸血学会雑誌 52(2): 198 第54回日本輸血学会総会 2006年6月 9-11日	医療機関において輸血後感染症の全数調査を実施したところ、輸血後陽転例はHBVで9例(0.9%)存在し、1例のみが輸血後B型肝炎と診断され、他の8例は感染晩期のHBVキャリアで再活性化が起こったと考えられた。HCVとHIVでは輸血後陽転例はみられなかった。	3
60121	2006/09/29	B型肝炎 C型肝炎	日本輸血学会雑誌 52(2): 199 第54回日本輸血学会総会 2006年6月 9-11日	日本の献血者におけるHBV NAT陽性者について解析したところ、20歳代ではHBV感染初期、50歳代、60歳代では感染晩期の陽性例が多かった。陽性数の高い地域は千葉県から愛知県までの太平洋側と大阪府であった。HBV Genotype Aは101例(女性1例)検出され、全国に広がる傾向が見られた。	4
60121	2006/09/29	B型肝炎 C型肝炎	日本輸血学会雑誌 52(2): 234 第54回日本輸血学会総会 2006年6月 9-11日	2005年に全国の医療機関から日本赤十字社へ報告された輸血感染症(疑い症例を含む)の現況とその傾向についての報告である。2005年輸血感染症症例(疑い症例を含む)報告数は12月22日現在260例で、内訳は、HBV:127、HCV:71、HEV:2、HIV:2、CMV:3、ヒトパルボウイルスB19:3、細菌:52であった。この内、輸血との因果関係が高いと評価した症例は、HBV:10例、HCV:1例、HEV:1例、ヒトパルボウイルスB19:3例であった。	5
60121	2006/09/29	C型肝炎	41st Annual Meeting of the European Association for the Study of the Liver 2006年4月26-30日	スペインの肝臓及び消化器専門クリニック数施設における急性C型肝炎の全報告例についてレトロスペクティブ疫学解析を行った。1998年から2005年の急性C型肝炎患者103例を対象とした。大部分の症例で感染に関連する唯一の立証されたリスクファクターは入院であった。	6

感染症定期報告の報告状況（平成18年9月1日～平成18年11月30日）

血対ID	受理日	感染症（PT）	出典	概要	
60158	2006/10/27	C型肝炎	Transfusion 2006; 46: 469-475	米国の5つの血液センターで1999年から2001年12月に供血した2,579,290例についてHCV抗体、NATデータ、ALT値および人口統計学的特徴について分析した。ALT値はRNA陽性の初回ドナーで有意に高い傾向があった。ウイルス血症寛解は白人の方がアジア系や黒人より有意に高かった。	7
60121	2006/09/29	E型肝炎	J Gen Virol 2006; 87: 949-954	日本固有のE型肝炎ウイルスの分子学的追跡を行った。日本で回収された遺伝子型3HEV24株および遺伝子型4HEV24株は、821nt RNAポリメラーゼ遺伝子フラグメントから成る系統樹で、外国株とは明らかに異なるクラスターを示した。ヌクレオチド置換速度から、日本固有HEVの先祖は、英国から日本へヨークシャ種のブタが輸入された1900年頃進入したと考えられた。遺伝子型3の進化は1920年代から始まり、遺伝子型4は1980年代から急速に広まった。日本におけるHEVの土着化と蔓延は豚肉摂食の大衆化と関連する。	
60121	2006/09/29	E型肝炎	J Med Virol 2006; 78: 473-475	2005年に、英国国内で感染したE型肝炎の症例が、バーミンガム市内の病院で8例発見された。E型肝炎は英国ではまれな疾患と考えられており、通常は流行地への渡航後に発症する。急性肝炎の患者すべてに、渡航歴とは無関係にHEVの可能性を考慮する必要がある。	
60176	2006/11/22	E型肝炎	肝臓 2006; 47: 384-391	わが国のE型肝炎の実態を明らかにする目的で、全国から総数254例のE型肝炎ウイルス感染例を集め、これを解析した。その結果、以下の知見を得た。1)HEVは全国に浸透している。2)感染者の多くは中高年（平均年齢約50歳）で、男性に多い。3)我国に土着のHEVの遺伝型は3型と4型である。4)年齢と肝炎重症度に相関がある。5)遺伝型は4型が顕在化率も重症化率も高い。6)発症時期が無季節性である。7)感染経路は、動物由来食感染が約30%、輸入感染が8%、輸血感染が2%、不明が約60%であった。	8
60123	2006/09/27	E型肝炎	肝臓 2006; 47(Supplement1): A168	今回われわれは血液感染のハイリスクグループである血友病患者におけるHEV抗体の陽性頻度を調査した。その結果、調査した血友病患者80例の内の13例（16.3%）がHEV抗体陽性であった。過去の研究では日本人の供血者におけるHEV抗体の陽性率は3.7%、透析患者で9.4%と報告されており、血友病患者におけるHEV血液感染の可能性が示唆された。また、他のウイルスマーカーの陽性率については、HEV抗体陽性例と陰性例では差は見られなかったが、年齢が高い方がHEV抗体陽性例の割合が高かった。	
60117	2006/09/22	E型肝炎	日本赤十字社 http://www.mhlw.go.jp/shingi/2006/01/dl/s0126-10e05.pdf	北海道赤十字血液センターで献血者のHEV保有状況を調べた。平成16年11月1日から平成17年10月31日にブタ、シカ等の生肉、生レバーの喫食歴のある献血者は298,790人中802人(0.28%)で、その血液検体からHEV-RNAが1例検出された。平成17年11月1日から12月31日に生肉、レバー等の喫食歴のある献血者は49,361人中13,835人（28.0%）で、HEV-RNAが5例検出された。平成17年1月1日から12月31日の試行的HEV20プールNAT検査での陽性率は約1万分の1であった。	
60123	2006/09/27	E型肝炎	日本輸血学会誌 2006; 520: 231	北海道地区において試験研究的に献血時にHEV関連問診を追加するとともに、HEV NATスクリーニングを実施し、問診の有効性とHEV感染の実態を調査した。結果は、HEV問診に該当したのは765名（0.3%）で、その内の1名（0.1%）にHEV RNAが認められた。HEV NATスクリーニング陽性者は20名（HEV問診該当者1名を含む）で、陽性率は1/11,090であった。陽性者の多くはALT値が正常でHEV抗体は陰性であった。道内の献血者のHEV　RNA陽性率は予想以上に高い。	

感染症定期報告の報告状況（平成18年9月1日～平成18年11月30日）

血対ID	受理日	感染症(PT)	出典	概要	
60174	2006/11/21	HIV	日刊薬業 第12105号 平成18年9月6日	日本人初のHIV-2感染者が確定された。男性は過去に西アフリカに渡航し、現地で輸血した経験があるため、これが感染経路と見られている。厚労省は、2型の検査も確実に行い、検査漏れがないよう、各都道府県に通知した。	9
60121	2006/09/29	HTLV	International Conference on Emerging Infectious Diseases 2006; Mar 19-22; Atlanta, Georgia. Abstracts #50	狩猟、屠殺、飼育を通して非ヒト霊長類(NHP)の血液と接触がある中央アフリカ人930名の血しょう検体を用いて、HTLV多様性を調べた。ウエスタンブロット法で陽性の13例から、PCRによりプロウイルスを増幅し、系統発生学的分析を行った。その結果、HTLV-3とHTLV-4と名づけた新しいウイルスの感染例(2例)が明らかになった。HTLV-3は、今までヒトでは見られなかったSTLV-3に属する。11例でマンドリル由来のものなど、多様なHTLV-1感染が見られた。	
60121	2006/09/29	ウイルス感染	Emerg Infect Dis 2005; 11: 1874-1881	米国において、ブタのノロウイルスについて調べるため、正常なブタの糞便275検体をRT-PCR法によりスクリーニングした。6例が陽性で、遺伝子配列分析の結果、ゲノグループIIの型と潜在的組み換え型が同定された。1つの遺伝子型は遺伝子的、抗原的にヒトノロウイルスと関連性があった。	
60121	2006/09/29	ウイルス感染	Eurosurveillance 2006; 11(4): 060420	2005年4月1日から2006年2月28日の間に、フランスで307例のチクングンヤ輸入例が同定された。平均年齢は47歳(7-81歳)であった。月別の輸入例数はレユニオンでの発生状況と関連が見られた。自発例は2006年3月に1例発生したが、輸入例患者をを看護した看護師で、インド洋への旅行歴はなく、血液の暴露による感染と考えられた。	
60148	2006/10/25	ウイルス感染	Eurosurveillance 2006; 11(8): 060810	2005年12月以降、チクングンヤウイルス感染のアウトブレイクがインドの8つの州で続いており、拡大するおそれがある。最も被害の大きい5つの州では896500例以上の疑い例が報告されている。北部の州からは1例も報告されていない。ヨーロッパの多数の国で輸入症例が報告されている。感染の拡大防止ならびに特異的な抗ウイルス薬とワクチンの開発が急務である。	10
60123	2006/09/27	ウイルス感染	J Med Primatol 2005; 34(S1): 333	サル泡沫状ウイルス(SFV)は非ヒト霊長動物で蔓延している非病原性感染症であるが、唾液を介して伝播すると考えられている。最近ヒトでの感染が報告された。SFVが血液を介して伝播するかを調べるため、SFV陰性アカゲザルに生物学的および遺伝的に異なったSFVに感染した2匹のアカゲザルの血液を輸血し、ウイルス感染および持続、抗体反応、臨床的変化をモニターした。接種後1年目の結果から、全血でSFVが伝播することがあることが示された。	
60116	2006/09/26	ウイルス感染	J Med Virol 2006; 78: 693-701	海洋起源のVesivirus感染がヒトでどの程度広がっているかを調べた。供血基準をクリアした供血者群、ALT値が高いため供血不可であった供血者群、非A-G肝炎患者群、および輸血または透析に関連した肝炎患者群由来の血清をVesivirusに対する抗体ならびにゲノムについて検査した。Vesivirusビリオンに対する血清陽性は、各々、12%, 21%, 29%, 47%であった。RT-PCRの結果、SMSV Vesivirusなどとの関連性が示された。感染がヒトに広くみられることが明らかになった。	

感染症定期報告の報告状況（平成18年9月1日～平成18年11月30日）

血対ID	受理日	感染症（PT）	出典	概要	
60121	2006/09/29	ウイルス感染	ProMED20060205-0040	インド洋西部でチクングンヤウイルスによる感染症が流行している。レユニオン島では2006年1月下旬の1週間だけで1万5千人増え、計5万人に達した。モーリシャス当局はウイルスを媒介するヒトスジシマカの駆除を決定した。	
60123	2006/09/27	ウイルス感染	ProMed20060225-0619	インド洋海域からフランス本土への帰国者の中にチクングンヤ感染者が発見された。フランス保健省によるとチクングンヤ熱は治療法もなく、ワクチンもなく、フランス領レユニオン島では77人が死亡し、現在も人口の約20%が感染している。	
60158	2006/10/27	ウイルス感染	Transfusion 2006; 46: 1352-1359	全血輸血により、サルfoamyウイルス（SFV）感染が起こるかをアカゲザルを用いて調べた。自然感染ザル2匹の全血を、各々、レトロウイルスを持たないサル2匹に輸血したところ、1匹のドナーからのレシピエントでは感染し、もう1匹のドナーからのレシピエントは感染しなかった。ヒトでのSFV輸血伝播の可能性が示された。	11
60110	2006/09/19	ウイルス感染	カナダ Public Health Agency of Canada 2006年5月26日	カナダ公衆衛生局は最近4例のカナダ人旅行者でチクングンヤ感染が原因と思われる疾患を確認した。これらの患者はレユニオン島などへ旅行し、2月から3月の初めに発症した。ヨーロッパでも帰国者による輸入例が報告されている。インド洋南西諸島で2005年3月から2006年4月22日までの間に公式に報告されたチクングンヤ感染例は3877例であるが、実際には255000例に達すると思われる。インドでは2005年12月以来、チクングンヤウイルスのアウトブレイクが報告され、2006年4月20日現在、153324例に達する。	
60110	2006/09/19	ウイルス感染	第80回　日本感染症学会総会・学術講演会	日本におけるヒトボカウイルス(HBoV)検出状況を調査した。2002年10月～2003年9月、2005年1月～7月の2シーズンに、小児下気道感染症患者318例から採取した鼻咽頭スワブより抽出したDNAをPCRし、塩基配列を決定した。318例中18例(5.7%)でHBoVが検出され、検出された患者の年齢は7ヶ月から3歳で、検出月は1月から5月に集中していた。HBoVは様々な呼吸器感染症の原因ウイルスになっていると推定された。	
60121	2006/09/29	コロナウイルス感染	Clin Infect Dis 2006; 42: 634-639	新規ヒトコロナウイルスHKU1は呼吸器及び腸疾患に関連する可能性があり、状態の不良な患者における持続性の無症候性感染との関連が考えられる。	
60117	2006/09/22	伝染性紅斑	Transfusion 2005; 45: 1811-1815	6ヶ月間にわたり血液疾患患者に投与された合計2123の血液製剤について、パルボウイルスB19DNAの有無をPCRにより調べた。その結果、21製剤(1%)が陽性であった。試験期間中114例の患者のうち14例がB19DNA陽性の血液成分を投与されたが、急性B19感染症を呈した患者はいなかった。	
60116	2006/09/26	パルボウィルス	Emerg Infect Dis 2006; 12: 151-154	米国で医薬品製造用血漿プールの検体においてPCR法によりパルボウイルス(PARV4)遺伝子の検出を行った。これらの血漿はヨーロッパと北アメリカで集められたものである。その結果、137プール中7例がPARV4およびPARV5に陽性であった。	
60158	2006/10/27	パルボウィルス	J Clin Virol 2006; 35: 407-413	血液プール中の感染性パルボウイルスB19を自動的に高速に検出するための方法を開発した。B19蛋白合成は免疫蛍光染色で検出され、ウイルスDNA合成はdot blot hybridizationと定量的PCRで検出された。調べられた細胞株の中ではUT7/Epo-S1がB19感染に最も感受性が高かった。	12

感染症定期報告の報告状況（平成18年9月1日～平成18年11月30日）

血対ID	受理日	感染症（PT）	出典	概要	
60121	2006/09/29	パルボウィルス	J Infect Dis 2006; 194: 154-158	ヒトパルボウイルスB19DNA（1.6x10^{8}IU/mL）を含むプール血漿の輸血後、B19 IgG陽性の患者では抗体価が19-39 IU/mLから50-100 IU/mLに上昇して再感染を防いだ。それに対し、陰性の患者では、1.6-2.2 x 10^{8}IU/mLのB19DNAの存在下で、プール血漿のIgGレベルが59.5IU/mLではB19の伝播とセロコンバージョンを防ぐのは不十分であることがわかった。	13
60123	2006/09/27	パルボウィルス	Thromb Haemost 2004; 92: 838-845	今まで、凝固因子製剤のパルボウイルスB19遺伝子型2による汚染は報告されていないので、市販されている21製剤の202ロットを、パルボウイルスB19遺伝子型1と遺伝子型2のDNAについてPCRにより調べた。遺伝子型1のDNAが、現在投与されているロットの77/181（42.5%）に、1980年代初めまで使用されたロットの17/21（81%）に検出された。遺伝子型2のDNAは、5/202（2.5%）に見出され、その全てが遺伝子型1のDNAに汚染されていた。	14
60174	2006/11/21	マラリア	AABB Weekly Report 2006; 12(32): 10	2006年9月8日付けのKorea Timesによると、交通事故後に輸血を受け、その後マラリアと診断された患者が、疑わしい血液の流通を防ぐことができなかった韓国赤十字を批判している。韓国赤十字は状況を知りながら、何の行動も起こさず、その結果、不適切な血液製品が流通し続けたとのことである。輸血によるマラリア感染で既に1名の患者が死亡している。	15
60122	2006/09/29	マラリア	CDC 2006年6月30日	米国疾病対策予防センターは、バハマのグレートエグズーマ島におけるマラリア発生を確認し、旅行者に対して抗マラリア薬の使用を勧告した。2006年6月29日現在、患者数は合計18例で、うち4例が旅行者である。全員Plasmodium falciparumに感染していた。	16
60122	2006/09/29	マラリア	ProMED-mail20060624.1758	キルギスタンの首都で79例のマラリア症例が登録された。予防手段を講じているにもかかわらず、流行が拡大中である。住民に問題を理解させるのが難しく、衛生疫学監視センターの職員は時々しか殺虫剤を散布できない。	17
60121	2006/09/29	ムンプス	AABB Association Bulletin #06-04 2006年4月26日	アイオワ州では2005年12月以来、おたふくかぜが大流行中で、2006年4月20日時点で、疑い例も含め、1000例以上がアイオワ公衆衛生部に報告されている。おたふくかぜの輸血による伝播に関する現在の知見に基づき、AABBの輸血伝播病委員会およびFDAは、血液収集施設が行うべき予防的アプローチを承認した。	18
60121	2006/09/29	ﾘﾝﾊﾟ性脈絡髄膜炎	N Engl J Med 2006; 354: 2235-2249	2003年12月及び2005年4月に固形臓器の移植を受けた2つの患者群の感染症について調べた。レシピエント全員（8名）の検体からリンパ性脈絡髄膜炎ウイルス（LCMV）が検出されたが、ドナー（2名）からは検出されなかった。2005年群のドナーはLCMVに感染したハムスターをペットとして飼っていたが、2003年群の感染源は不明であった。レシピエント8例中7例は移植後9日から76日で死亡した。	19
60148	2006/10/25	変異型クロイツフェルト・ヤコブ病	AABB Weekly Report 2006年7月21日	アイルランド輸血サービスは、CJDの病因となるプリオンを供血血液から除去するために開発された新しい装置を1年間使用した後、試用の中止を決定した。血液サービスは昨年、そのフィルター装置を購入したが、十分な効果が得られず、CJDプリオンは捕捉されずに通過し、供血中に混入する可能性があるためである。	20

感染症定期報告の報告状況（平成18年9月1日～平成18年11月30日）

血対ID	受理日	感染症（PT）	出典	概要	
60121	2006/09/29	変異型クロイツフェルト・ヤコブ病	Blood 2006; 107: 3907-3911	PrPCは全身の多数の組織に存在し、血小板に大量に存在する。静止血小板ではα顆粒膜上に存在することが知られているが、その生理学的機能は不明である。血小板中のPrPcの局在を調べたところ、血小板が活性化すると、血小板表面上にPrPCが一時的に発現し、続いて、微小胞およびエキソソーム上への放出が起こることが明らかとなった。血小板由来エキソソーム上にPrPCが存在するということは、血中でのPrPC輸送および細胞間伝播におけるメカニズムを示唆する。	21
60121	2006/09/29	変異型クロイツフェルト・ヤコブ病	BMJ 2006; 332: 1186-1188	1996年から1999年に、手術時に20-29歳であった患者から得られた虫垂および扁桃12674検体のうち、病原体プリオンに陽性染色であった3例(虫垂)について、プリオン蛋白の遺伝子型分析を行った。3検体中2例で分析が可能であり、両者ともプリオン蛋白遺伝子(PRNP)コドン129のValがホモ接合体であった。今まで、vCJD患者は、Met/Valのヘテロである医原性の1例を除いて全て、PRNPのコドン129がMetのホモ接合体であり、Valホモ接合体がvCJDに対し感受性があることが初めて示された。	22
60123	2006/09/27	変異型クロイツフェルト・ヤコブ病	BMJ Online doi:10.1136/bmj.38804.511644.55	1996年から1999年に、手術時に20-29歳であった患者から得られた虫垂および扁桃12674検体のうち、病原体プリオンに陽性染色であった3例(虫垂)について、プリオン蛋白の遺伝子型分析を行った。3検体中2例で分析が可能であり、両者ともプリオン蛋白遺伝子(PRNP)コドン129のValがホモ接合体であった。今まで、vCJD患者は、Met/Valのヘテロである医原性の1例を除いて全て、PRNPのコドン129がMetのホモ接合体であり、Valホモ接合体がvCJDに対し感受性があることが初めて示された。	
60117	2006/09/22	変異型クロイツフェルト・ヤコブ病	CDR Weekly 2006; 16(6)	英国で、献血の20ヶ月後にvCJDを発症したドナーからの血液(赤血球)を輸血された患者が、8年後にvCJDと診断された。これは英国において輸血伝播によると思われるvCJD感染の3症例目である。	
60176	2006/11/22	変異型クロイツフェルト・ヤコブ病	Future Virol 2006; 1: 659-674	血液製剤の製造工程におけるプリオンの除去に関する総説である。プリオン除去のための個々の製造工程は、実際の製造条件を実験室での条件にスケールダウンさせ、確立されているスクレイピー株をモデル系として用いて通常は評価されている。しかしながら、血液中のプリオンタンパクの存在形態が不明なので、評価実験のためのスパイク材料としてのプリオンの調製方法は注意深く考慮しなければならない。現在のところ、エタノール分画、PEG 分画、カラムクロマトグラフィー、ウイルス除去膜およびデプスフィルターでの濾過が有効とされている。	23
60134	2006/10/18	変異型クロイツフェルト・ヤコブ病	Guidance for Industry (DRAFT GUIDANCE) U.S. Department of Health and Human Services Food and Drug Admini-stration Center for Biologics Evaluation and Research August 2006	古典的CJDの潜伏期間は38.5年であり、vCJDの潜伏期間も非常に長いことが示唆されている。また、未確認ではあるが恐らくかなりの数の血液ドナーが、欧州におけるBSE激増中にフランスで感染した可能性がある。これらのことから、FDAは1980年以降フランスで血液又は血液成分の輸血を受けた者からの供血を無期限に停止するという予防策の導入をガイダンス案として発表した。	24

感染症定期報告の報告状況（平成18年9月1日～平成18年11月30日）

血対ID	受理日	感染症（PT）	出典	概要	
60158	2006/10/27	変異型クロイツフェルト・ヤコブ病	Haemophilia 2006; 12(Suppl. 1): 16-20	vCJDが英国の血友病患者の臨床に与えた影響について述べた。2003年に輸血伝播vCJDによる最初の死亡例が報告され、2004年に輸血伝播vCJDのリスクがある全ての患者に通知することが決定された。2005年4月現在、A型およびB型血友病患者は全員、遺伝子組換え凝固因子を投与されている。	25
60158	2006/10/27	変異型クロイツフェルト・ヤコブ病	Haemophilia 2006; 12(Suppl. 1): 26-28	血友病の管理における新興病原体についての討論。英国ではvCJDが特に大きな脅威であるが、白血球除去により完全に伝播を防ぐことはできない。また遺伝子組換え製剤による治療が、全てのウイルス伝播リスクをなくすわけではないことなどが論じられている。	26
60158	2006/10/27	変異型クロイツフェルト・ヤコブ病	Haemophilia 2006; 12(Suppl. 1): 8-15	vCJDは食物でBSEに暴露することで発生するとされていたが、輸血や血液製剤によっても伝播することが報告された。今までのヒトでの発症例は全てヒトプリオン遺伝子のコドン129がメチオニンホモ型のヒトであり、、感染していても発症しない無症候性キャリアがいるおそれがある。非侵襲性の血液検査の開発が急務である。	27
60126	2006/09/27	変異型クロイツフェルト・ヤコブ病	J Neurol Neurosurg Psychiatry 2006; 000: 1-3	1970年～2003年にヒト硬膜に関連したCJD7例が英国で確認された。手術後発病までの期間は平均93ヶ月（45～177ヶ月）であった。さらに、世界で初めて、ブタ硬膜片レシーピエントでCJD1例を確認した。これらの症例の臨床的、病理学的特徴について述べている。	28
60123	2006/09/27	変異型クロイツフェルト・ヤコブ病	Lancet 2006; 367: 2068-2074	1996年7月から2004年6月までに11人のクールー病患者を確認したが、全員がSouth Foreに住んでいた。患者は全員、1950年代後半に食人習慣が中止される前に生れていた。推定された潜伏期間は、最小で34年から41年の範囲であったが、男性における潜伏期間は39年から56年の範囲と考えられ、更に最長で7年長かった可能性もある。プリオン遺伝子の分析によって、殆どのクールー病の患者は、潜伏期間の延長とプリオン病への耐性に関係する遺伝子型であるコドン129がヘテロ接合体であることが明らかとなった。	29
60121	2006/09/29	変異型クロイツフェルト・ヤコブ病	Lancet 2006; 367: 874	2004年2月に50歳の日本人男性がCJDサーベイランス委員会に報告された。男性は英国およびフランスに滞在歴があった。2001年6月に発病し、2003年1月には脳脊髄液は14-3-3蛋白質陽性であった。PrP遺伝子解析では変異は見られなかった。2003年12月にはMRIと脳波より、sCJD可能性例と診断された。2004年12月に死亡し、剖検によりvCJDと診断された。日本初のvCJD確定例である。	
60123	2006/09/27	変異型クロイツフェルト・ヤコブ病	Lancet Neurol 2006; 5: 393-398	マウスPrP遺伝子の置換によってヒトまたはウシのコドン129遺伝子型（MM、MV、VV）のPrP蛋白を発現するマウスを作製し、BSE又はvCJDを接種し、疾患の臨床的及び病理学的な徴候を評価した。その結果、BSEはウシの系には感染したが、ヒトの系には感染しなかった。対照的に、vCJDはヒトの3つの系全てに感染したが、各々の遺伝型で病理学的特徴、感染効率が異なった。MMは感染効率が高く、病理学的特徴および臨床症状が早く発現した。、VVは感染効率が最も低く、発現までの期間が長かった。	

感染症定期報告の報告状況（平成18年9月1日～平成18年11月30日）

血対ID	受理日	感染症(PT)	出典	概要	
60123	2006/09/27	変異型クロイツフェルト・ヤコブ病	PLoS Pathogens 2006; 2: e32	土壌ミネラルと病原性プリオン蛋白(PrPSc)の相互作用を検討することによって、土壌がTSE蓄積体として提供される可能性を調べた。その結果、2種類の粘土ミネラル、石英および4種類の全土壌サンプルにPrPScが吸着し、感染性も維持されることが明らかとなった。我々の研究結果は、土壌環境に入ったPrPScは生物に利用できる形態で維持され、プリオン病の動物感染を永続させるとともに、他の種をこの感染性病原体に曝露させる可能性があることを示している。	
60123	2006/09/27	変異型クロイツフェルト・ヤコブ病	Robert Koch Institut/ Voten des Arbeitskreises Blut/ Votum 33	2006年1月11日の血液専門委員会第61回会議で可決されたvCJDに関するドイツ連邦保健省血液専門委員会の通達である。まだ使用されていない血液製剤によるvCJDの感染を防止し、感染の可能性のある供血を予防し、感染が生じた場合の解明の手順を定めた。	
60121	2006/09/29	変異型クロイツフェルト・ヤコブ病	Science 2006; 311: 1117	慢性消耗病(CWD)のシカの骨格筋中に感染性プリオンが含まれているかどうかを、シカのプリオンを発現するトランスジェニックマウスにおいて検討した。CWDに感染したシカの骨格筋抽出物を脳内に接種したトランスジェニックマウスは360～490日後に、脳抽出物を接種した群は230～280日後に、進行性神経症状を呈し、これらのマウスの脳にはPrPscが検出された。正常シカの抽出物を接種した対照群では発病しなかった。	
60121	2006/09/29	変異型クロイツフェルト・ヤコブ病	Science 2006; 313: 92-94	TSEの前兆期に、スクレイピーに感染させたハムスターの血液中のPrPScをPMCA (protein misfolding cyclic amplification)法を用いて生化学的に検出した。潜伏期間の初期には、おそらく血液中に検出されたPrPScは末梢でのプリオンの複製に由来していると思われる。感染しているが発症していない動物の血液中のプリオンを生化学的に検出することができるということは、TSEの非侵襲的早期診断を期待させる。	30
60121	2006/09/29	変異型クロイツフェルト・ヤコブ病	Sunday Herald 2006年3月5日 http://www.sundayherald.com/54442	vCJD専門家が、ヒツジとヤギにおける非定型スクレイピーの危険性を警告している。ヒトに感染するおそれがあるため、、現在18月齢以上のヒツジに行われているTSE検査を、もっと若いヒツジに対しても行うように求めている。	
60121	2006/09/29	変異型クロイツフェルト・ヤコブ病	The Guardian 2006年5月2日	英国は、1990年代に輸出された英国製の血液製剤からのvCJD感染の危険性について、輸出先の14ヵ国に連絡を行った。輸血を介したvCJD感染は英国では3例報告されており、未発症の感染者からの供血により引き起こされる災害の「第二の波」が懸念される。最も危険性の高いブラジルとトルコや、ブルネイ、アラブ首長国連邦、インド、ヨルダン、オマーン、シンガポールに予防措置をとるよう勧告した。	31

感染症定期報告の報告状況(平成18年9月1日～平成18年11月30日)

血対ID	受理日	感染症(PT)	出典	概要	
60117	2006/09/22	変異型クロイツフェルト・ヤコブ病	The Guardian 2006年5月2日 http://www.guardian.co.uk/frontpage/story/0,,1765531,00.html	英国は、1990年代に輸出された英国製の血液製剤からのvCJD感染の危険性について、輸出先の14ヵ国に連絡を行った。輸血を介したvCJD感染は英国では3例報告されており、未発症の感染者からの供血により引き起こされる災害の「第二の波」が懸念される。最も危険性の高いブラジルとトルコや、ブルネイ、アラブ首長国連邦、インド、ヨルダン、オマーン、シンガポールに予防措置をとるよう勧告した。	
60158	2006/10/27	変異型クロイツフェルト・ヤコブ病	Transfusion 2006; 46: 652-658	血漿分画時にTSE物質を除去する研究のために、スクレイピーに感染した脳から水溶性の感染性検体を調整した。263Kスクレイピー感染ハムスターの脳を10%PBS中に懸濁し、低速遠心分離後、更に上澄みを超遠心(220000xg)した。得られた上澄みは感染性を有し、しかもPrPTSEの凝集体を全くまたはごくわずかしか含有しなかった。	32
60123	2006/09/27	変異型クロイツフェルト・ヤコブ病	英国保健省 Press Statement 2006年2月9日	輸血関連の新しいvCJD1症例が最近診断された。患者は、献血をした約20ヶ月後にvCJD症状を呈したドナーからの輸血を受け、輸血後約8年でvCJDを発症した。患者はまだ生存している。本症例は3例目の輸血によるvCJD感染例である。	
60121	2006/09/29	変異型クロイツフェルト・ヤコブ病	英国保健省 Press Statement http://www.hpa.org.uk/hpa/news/articles/press_releases/2006/060209_cjd.htm	輸血と関連したvCJDの新たな症例が見つかった。患者は、供血後20ヶ月でvCJDを発症したドナー由来血液の輸血を受け、その約8年後にvCJDを発症した。この患者は存命中で、国立プリオン病院の医師による治療を受けている。この症例は輸血関連vCJD伝播としては3例目であり、vCJDが輸血によってヒト―ヒト感染しうることを示す新たな証拠となるだろう。これら3例は、血漿分画製剤の投与ではなく、すべて血液成分の輸血と関連している。	
60174	2006/11/21	変異型クロイツフェルト・ヤコブ病	厚生労働省 平成18年8月24日	平成18年8月23日に開催された薬事・食品衛生審議会血液事業部会安全技術調査会において、ヒト胎盤エキス(プラセンタ)注射剤使用者に対する献血制限措置を日本赤十字社が実施することが了承された。	33
60158	2006/10/27	ウイルス感染	Haemophilia 2006; 12(Suppl. 1): 3-7	1992年以来、米国の医学研究所(IOM)は、「新興感染症は、新型の、または再興する、または薬剤耐性の感染症であり、ヒトでの発生率が過去20年で増加しているか、近い将来増加するおそれがある疾患」と定義している。現在、トリインフルエンザ、ウエストナイルウイルス、重症急性呼吸器症候群(SARS)コロナウイルスの3つが注目されている。供血中の感染物質の存在は、血友病患者の治療に使われる血液や血液由来製品の安全性に重大な影響を与える。病原体の検出と除去が重要である。	34
60110	2006/09/19	肝炎	J Infect Dis 2006; 193: 1089-1097	非特異的PCRを行った後、染色体由来配列を除去することにより、非A-E肝炎患者の血清から、外来DNA断片が得られた。これらの内の一つをNV-Fと名づけたが、部分的オープンリーディングフレームを含み、非A-E肝炎患者69例中17例(24.6%)に検出された。NV-F陽性患者65例中49例(75.4%)の血清中に抗NV-F抗体が検出された。また免疫蛍光分析により、抗原は患者の肝細胞に存在することが明らかとなった。NV-Fはヒト肝炎に関連する新規の1本鎖DNA断片である。	

感染症定期報告の報告状況（平成18年9月1日～平成18年11月30日）

血対ID	受理日	感染症（PT）	出典	概要	
60173	2006/11/15	結核	WHO 2006年9月5日	WHOは病原性が強く、致死性の結核の世界的な拡大防止の強化および措置を求めた。6クラスの第2選択薬の3クラス以上に耐性のある多剤耐性結核（XDR-TB)は世界の様々な地域で確認されており、特に旧ソビエト連邦やアジアで多い。また南アフリカではXDR-TBでHIV陽性である患者群で極めて高い死亡率が確認されている。	35
60122	2006/09/29	細菌感染	Transfusion 2006; 46: 305-309	血小板供血歴の長い、無症候性の58歳男性由来の血小板が単球症リステリア陽性であった。パルスフィールドゲル電気泳動パターンはCDCデータベース中の他の2例の単球症リステリア分離株と一致したが、疫学的な関連性は見られなかった。	
60122	2006/09/29	細菌感染	Transfusion 2006; 46: 476-485	オランダで2002年11月に全国統一の皮膚消毒法（70%イソプロピルアルコールを用いたダブルスワブ消毒）が導入された。2002年から2003年に、プールされた軟層由来の濃縮血小板113,093例をスクリーニングしたところ、新消毒法導入後の初回陽性は0.85%で、導入前の0.95%と比べ、わずかな減少あった。初流血除去バッグを使用していた施設では細菌汚染の頻度は有意に低く、新消毒法導入前は0.5%、導入後は0.37%であった。アフェレーシス濃縮血小板は8000例中24例(0.3%)が初回陽性であった。	
60143	2006/10/18	鳥インフルエンザ	Arch Virol 2006 Published online Feb 26, 2006	2004年初頭、日本で発生した高病原性トリインフルエンザで死亡したニワトリから単離されたA/chicken/Yamaguchi/7/04(H5N1)ウイルスを、ニワトリ、ウズラ、セキセイインコ、コガモ、マウス、ミニブタに経鼻で接種し、実験的に感染させることにより病原性を評価した。このウイルスは調べられた全てのトリで高病原性を示し、ニワトリは接種後4日以内に6例全て死亡し、ウイルスは呼吸器、肝臓、腎臓、大腸、脳から検出されたが、血液からは検出されなかった。マウスは感染に感受性はあるが、致死率は低かった。	36
60158	2006/10/27	鳥インフルエンザ	ECDC Technical Report 2006年6月1日	ヨーロッパで発生している高病原性トリインフルエンザウイルス、特にA型H5N1による公衆衛生学的リスクについて論じている。ヒトの健康へのリスクとしては、トリウイルスによる直接感染、ならびに新しいA型インフルエンザ株の発生がある。感染予防のためには家禽および野鳥の調査、獣医と医療との連携、適切な情報の提供などが必要である。	37
60111	2006/09/29	鳥インフルエンザ	Emerg Infect Dis 2006; 12: 1041-1043	タイで2005年11月28日にトリインフルエンザを発病し、12月7日に死亡した5歳の少年の血液検体を調べた。RT-PCRにより、血漿はH5N1インフルエンザウイルス陽性であった。ウイルスを分離し、遺伝子配列を決定したところ、A/Thailand/NK165/05 accession no. DQ372591-8であった。ヘムアグルチニンとノイラミニダーゼ遺伝子について系統遺伝学的分析を行ったところ、2004年初めにタイで発生した野鳥のインフルエンザウイルスの特徴と同じであった。	
60121	2006/09/29	鳥インフルエンザ	WHO http://www.who.int/csr/don/2006_02_21b/en/index.html	トリインフルエンザの流行がアジア、アフリカ、ヨーロッパの国々に広がっている。2006年2月以降に初めてトリのH5N1感染を報告した国は、イラク、ナイジェリア、アゼルバイジャン、ブルガリア、ギリシャ、イタリア、スロベニア、イラン、オーストリア、ドイツ、エジプト、インド、フランスの13カ国にのぼる。	

別紙様式第2-1

No. 25

医薬品 研究報告 調査報告書

識別番号・報告回数			報告日	第一報入手日	新医薬品等の区分	機構処理欄
				2006. 7. 18	該当なし	

一般的名称	(製造承認書に記載なし)	研究報告の公表状況	Transfusion. 2006 Jul;46(7):1256-8. No abstract available. Allain JP, Reesink HW, Lucey C.	公表国 英国
販売名(企業名)	合成血「日赤」(日本赤十字社) 照射合成血「日赤」(日本赤十字社)			

研究報告の概要

○B型肝炎ウイルス陽性となった供血者と血液の管理に関するヨーロッパの考え方

2005年7月21日、米国FDAの血液製剤諮問委員会(BPAC)の会合が開かれ、「核酸増幅検査(NAT)でB型肝炎ウイルス(HBV)陽性となった供血者と血液の管理」に関して勧告された。一部の血液センターでは、B型肝炎表面抗原(HBsAg)とB型肝炎コア抗原に対する抗体(HBc抗体)検査に加えてHBV NAT検査の実施を選択した。供血者のリエントリーについては基準の検討中である。

ヨーロッパ北西部ではHBVの有病率は低く(1%未満)南部では高い(5~15%)。南東部ではジェノタイプDが多く、北西部ではジェノタイプAが多く見られる。有病率の低い国では抗HBcスクリーニングが導入されており、有病率の高い南欧の国ではHBV NATが導入される傾向にある。感染の広がっていない国ではどちらも実施されていない。

HBVの感染ルートはほとんどが性感染や静注薬物乱用であり、HBV NATで検出されるのは、HBsAgが検出される前のウインドウ期の症例およびオカルト感染症例の双方である。人口移動によってHBVジェノタイプの種類が多様化し、感染リスクが高まることも指摘されている。

オカルトHBV症例の中で、HBs抗体陽性のものは感染しないが、HBs抗体陰性であれば感染性があると考えられる。HBV DNA、HBc抗体陽性でHBs抗体レベルが100IU/L以上の場合は供血を続けることができるというリエントリー・アルゴリズムが検討されている。HBs抗体陰性のオカルトHBVキャリアでは、DNA検査の結果に誤りがあったと証明されない限り、リエントリーは認められない。

効果的なHBV NATスクリーニングシステムと症例の積み重ねの経験のみが、この補完検査アルゴリズムが血液の安全性と供血者の管理の向上にとって最善、実用的、決定的なものであるかどうかを教えてくれるだろう。

報告企業の意見	今後の対応
B型肝炎ウイルス陽性となった供血者と血液の管理に関して、ヨーロッパではHBV DNA、HBc抗体陽性でHBs抗体レベルが100IU/L以上の場合は供血を続けることができるというリエントリー・アルゴリズムが検討されているとの報告である。	日本赤十字社では、HBVについて20プールでスクリーニングNATを行い、陽性血液を排除している。HBV感染に関する新たな知見等について今後も情報の収集に努める。

使用上の注意記載状況・その他参考事項等

合成血「日赤」
照射合成血「日赤」

血液を介するウイルス、細菌、原虫等の感染
vCJD等の伝播のリスク

MedDRA/J Ver.9.0J

COMMENTARY

A European perspective on the management of donors and units testing positive for hepatitis B virus DNA

On July 21, 2005, the US FDA's Blood Products Advisory Committee (BPAC) met to advise FDA on "Management of Donors and Units that Test Positive for Hepatitis B Virus (HBV) DNA by Nucleic Acid Tests (NAT)." The best and most complete record of the meeting is the transcript, slides, and accompanying material.[1]

With the approval of the COBAS Ampliscreen HBV test, some US centers have opted to test for HBV NAT in addition to hepatitis B surface antigen (HBsAg) and antibody to hepatitis B core antigen (anti-HBc), which are both required for whole-blood donations (source plasma omits anti-HBc testing).[2] The algorithm does not change previous FDA deferral guidance, but the reentry of donors who may have a false-positive NAT test is under regulatory consideration. (Tables 1 and 2 in Reference 2 summarize the deferral algorithm for whole blood and source plasma.[2]) The algorithm follows three main principles:

1. Any HBsAg repeatedly reactive donor (initial screen positive with at least one of two repeat tests positive) that is confirmed by neutralization is permanently deferred.
2. Any NAT-positive and anti-HBc repeat-reactive donor (both lacking a licensed confirmatory test) is permanently deferred (two positive screening tests employing different laboratory methods).
3. Any NAT-positive HBsAg repeat-reactive donor without neutralization confirmation may be reentered.

Reentry can be evaluated at 6 months by the donation facility by retesting the screening panel (the NAT test would be single unit, not pooled) without a blood unit donation to protect against accidental release. If testing before the 6-month point has been performed for medical or donor notification, any NAT test positive permanently defers the donor.[2] If reentered, normal screening would be done on the next donation, thus providing two testing opportunities of the donor's blood following a deferral.

Based on the scientific data, BPAC unanimously agreed with FDA's proposal that

1. A donor of whole blood and blood components for transfusion who tests HBV NAT positive, anti-HBc nonreactive, and HBsAg nonreactive or HBsAg repeatedly reactive and/or not confirmed by neutralization may be reentered, if after a minimum period of 6 months, a sample from the donor tests negative for HBV DNA by individual-donation NAT, nonreactive for anti-HBc, and nonreactive for HBsAg.
2. A donor of source plasma for further manufacture into plasma derivatives who tests HBV NAT positive and HBsAg nonreactive or HBsAg repeatedly reactive and/or not confirmed by neutralization may be reentered, if after a minimum period of 6 months, a sample from the donor tests negative for HBV DNA by individual-donation NAT and nonreactive for HBsAg.

BPAC did not propose or approve any alternative approaches that the FDA should consider.[1] This commentary will provide a European viewpoint, with the *Guidelines for the Blood Transfusion Services in the UK*, 7th Edition, cited as a background reference.[3]

COMMENTARY: THE HBV DNA SITUATION IN THE EUROPEAN UNION

HBV epidemiology in Europe is characterized by a northwest to southeast shift in infection prevalence and genotype distribution. There is a low prevalence of HBV in the northwest (<1%) and higher in the south (5-15%), with genotype D more prevalent in the southeast and genotype A in the northwest.[4] As a result of the differences in prevalence, approaches to transfusion safety vary. In countries of relatively low prevalence such as France, Germany, or Ireland, anti-HBc screening has been introduced; in other countries, such as Greece, Italy, Spain, and Portugal, with higher prevalence, screening for anti-HBc would defer an unacceptable number of donors and blood banks are inclined to implement HBV-NAT. In most low endemic countries, neither anti-HBc nor NAT has been implemented for a variety of reasons including lack of cost-efficiency.

To date, there is no consensus in the EU or at national levels regarding HBV NAT except in Poland, where it was made mandatory in 2005 to test in either individual plasma samples or pools of no more than 24 donations, depending on the test manufacturer. Although not mandatory in Germany, Austria, and Luxembourg, some blood

These authors do not represent the opinions of any agency or organization. No official support or endorsement of this article should be inferred.

TRANSFUSION 2006;46:1256-1258.

banks in these countries have implemented HBV NAT in pools of various sizes, utilizing in-house or commercial assays, in which the loss of sensitivity is often compensated by a concentration step by ultracentrifugation.

The CE marking of the GenProbe/Chiron TMA triplex for HCV, HIV, and HBV genomes in 2004, called Ultrio, has somewhat modified the blood centers' attitude with regard to HBV NAT, particularly in those areas of southern Europe where HBV infection is prevalent but anti-HBc testing impractical. In these countries, HCV NAT is mandatory and HIV NAT rapidly spreading. A desire to ensure blood safety suggested more attention to HBV and the cost increase from the duplex (Procleix) to the triplex (Ultrio) appeared to be acceptable.

The epidemiology of HBV in southern Europe has changed with the widespread use of HBV vaccination, either in infants or in older children, beginning in the mid-1980s. These infant immunization programs have not yet significantly affected blood donors. HBV infection routes are mostly sexual or through intravenous drug abuse. As a result, the intended targets of HBV NAT are both incident cases in the pre-HBsAg window period and HBsAg-negative late stages of chronic or recovered infection (occult HBV infection is defined as a carrier of HBV DNA without detectable HBsAg irrespective of the anti-HBc and anti-HBs status).[5] In both cases, but more so in occult infection, DNA levels are very low, defeating the pooling strategies designed for HCV and HIV NAT. The proponents of HBV DNA screening also point out that the increasing influx of workers from areas of high HBV endemicity and their families will progressively diversify the spectrum of HBV genotypes and increase the risk of HBV in the donor pool. Overall, while centers in Germany, Austria, and Luxembourg continue pool testing, individual blood centers of southern Europe have mostly chosen individual donation screening (80%) or testing on small pools of eight samples (20%). No data have been published yet since screening started in 2005. Unpublished data suggest, however, that occult HBV infections are considerably more frequent than window-period cases. Health authorities in the EU and in individual countries are probably awaiting these data to define a policy that is likely to be heterogeneous depending on member state epidemiology.

In the European discussion forum, the position taken by the FDA is interesting but applies to donated blood screened for anti-HBc. The European situation more frequently tests donors without anti-HBc, although screening for anti-HBc is being considered as a strategy to avoid NAT and still be able to defer occult HBV carriers with detectable levels of anti-HBc. Dealing with three assays (HBsAg, anti-HBc, and HBV DNA) has the considerable advantage of assuming that two positive markers would be confirmatory and direct the permanent deferral of the donor. In European countries where NAT will be implemented without anti-HBc, the issue raised by the BPAC panel of an absence of licensed confirmatory HBV DNA assay will not be a factor since both Ultrio and Roche COBAS Ampliscreen HBV DNA are CE-marked. Either assay can be used for screening; the alternate can be used for confirmation.

Moreover, in countries where anti-HBc testing is introduced, it is common practice to measure the anti-HBs titer to determine the recovery status of the donor. In Germany, an algorithm is being considered to reinstate HBV NAT–negative anti-HBc–positive donors on the basis of an anti-HBs titer. Among occult HBV cases, those with anti-HBs (recovered) are unlikely to be infectious while those without anti-HBs (anti-HBc only) may be infectious. Examples of HBV transmission by transplanted livers from anti-HBs–positive donors, however, suggest caution when anti-HBc– and anti-HBs–positive blood components, even with titers of greater than 100 IU per L, are transfused to immunodeficient recipients. Nearly 50 percent of the transfused blood in Western Europe is given to recipients with some level of immunodeficiency. As a result the proposed algorithm being anti-HBc instead of HBV NAT is that every anti-HBc–positive sample be tested for both anti-HBs and HBV DNA. Donors HBV DNA–positive would be permanently deferred but those DNA negative with anti-HBs levels of at least 100 IU per L could continue donating. This prudent attitude is not necessarily endorsed by all European blood services or regulatory agencies. Where HBV NAT is implemented, detection and titration of anti-HBs would be useful to counsel donors, an anti-HBs titer of at least 100 IU per L strongly suggesting noninfectivity (see Fig. 1). This algorithm is hypothetical and to our knowledge not implemented anywhere in Europe.

For occult HBV without anti-HBs (anti-HBc only or DNA only), reentry is not an option unless the DNA result can be proven erroneous. We remain concerned that these donors may be infectious. There are a few reported cases of infectivity by transfusion and many of infectivity by transplanted organs from donors with the anti-HBc–only profile. Some cases might carry anti-HBs at the viral surface without detectable circulating anti-HBs, and those might be revealed by dissociation procedures. Others, and probably the most frequent, correspond to the tail end of chronic carrier state at the nonreplicative phase and are more likely infectious. This area remains problematic, and detailed characterization of the virus and solid clinical data are critically needed.

Although contamination has been raised as a source of difficulty for interpretation of positive tests, going back to the original units was not recommended. In Europe, it is likely that both returning to the collected unit and accessing the mandatory archived samples will be authorized. In Europe, unlike the United States, it is mandatory that a sample from all tested blood donations be kept for 2 years or more, depending on the country. This sample is

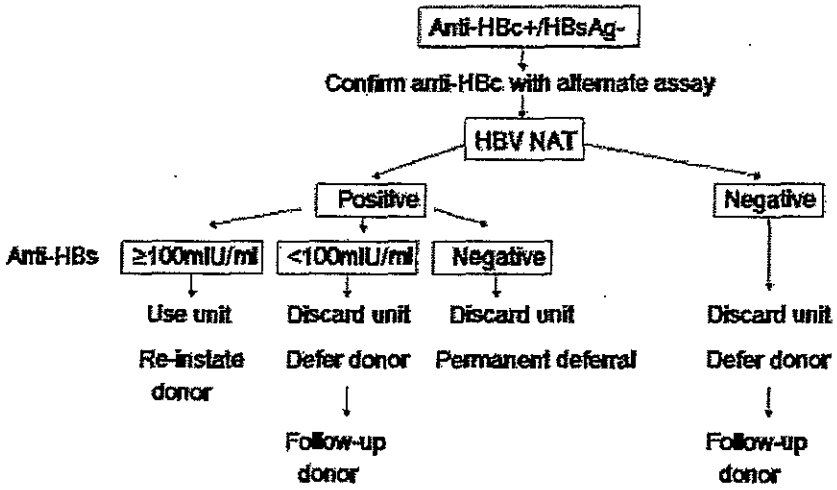


Fig. 1. Proposed European algorithm for blood donors anti-HBc–positive and HBsAg-negative.

intended for studies related to potential or emerging infectious agent transmission by transfusion. The alternative method of HBV diagnosis confirmation provided by the comparison of sequences will be difficult to implement for two reasons: one is that even in the most variable pre-S-S region, identical sequences within the same genotype are often found; the second is that when dealing with a low viral load, this method has a high failure rate.

The last and probably the most relevant issue is that a sample from an individual with a very low viral load might well be negative 6 months after deferral due to fluctuation of DNA level in occult infections.[6] Reinstatement of a NAT-positive donor on the basis of a negative result in a second test of the index donation or in a follow-up sample at 6 months tends to become "a lottery." Many Mediterranean blood banks that use HBV NAT on individual donations have started to confirm NAT reactivity not only with alternate assays, but also with duplicate or multiple repeat NAT screening tests. This procedure enables them to minimize the risk of missing low-level HBV DNA carriers and alerts them in time to save the unit for HBV resolution testing, which involves nucleic acid extraction from larger plasma volume and serological assays (N. Lelie, personal communication).

On both sides of the Atlantic, only experience with efficacious HBV NAT screening systems and the accumulation of yield case data will tell whether a given supplementary test algorithm is optimal, practical, and critical for improving blood safety and donor management.

Jean-Pierre Allain, MD, PhD
University of Cambridge
Cambridge, UK
Henk W. Reesink, MD, PhD
Department of Gastroenterology and Hepatology
Sanquin Blood Supply Foundation and Academic Medical Center (AMC)
Amsterdam, the Netherlands
Charles Lucey, MD, JD, MPH
21st Century Health Concepts Assoc.
Houston, TX

REFERENCES

1. Transcript, 83rd Meeting of: The Blood Products Advisory Committee [monograph on the Internet]. Rockville (MD): Food and Drug Administration, Center for Biologics Evaluation and Research; 2005 [cited 2006 Mar 23]. Available from: http://www.fda.gov/ohrms/dockets/ac/05/transcripts/2005-4164t1.htm
2. Topic I: FDA's current considerations on the management of whole blood and source plasma donors and units when a donor tests positive for hepatitis B virus (HBV) DNA by a nucleic acid test (NAT) [monograph on the Internet]. Gaithersburg (MD): Blood Products Advisory Committee; 2005 [cited 2006 Mar 23]. Available from: http://www.fda.gov/ohrms/dockets/ac/05/briefing/2005-4164B1_01.doc
3. Guidelines for the blood transfusion services in the UK [monograph on the Internet]. London: UK Blood Transfusion Services; 2005 [cited 2006 Mar 23]. Available from: http://www.transfusionguidelines.org.uk/index.asp?Publication=RB
4. Kidd-Ljunggren K, Miyakawwa Y, Kidd AH. Genetic variability in hepatitis B viruses. J Gen Virol 2002;83:1267-80.
5. Allain JP. Occult hepatitis B virus infection: implications in transfusion. Vox Sang 2004;86:83-91.
6. Dreier J, Kroger M, Diekmann J, Gotting C, Kleesiek K. Intermittent HBV viremia in an anti-HBc and anti-HBs-positive blood donor. Transfus Med 2005;15:65-6.

別紙様式第2-1

No. 26

医薬品 研究報告 調査報告書

識別番号・報告回数			報告日	第一報入手日	新医薬品等の区分	機構処理欄
				2006. 6. 20	該当なし	

一般的名称	(製造承認書に記載なし)	研究報告の公表状況	公表国
販売名(企業名)	合成血「日赤」(日本赤十字社) 照射合成血「日赤」(日本赤十字社)	佐竹正博. 第54回日本輸血学会総会; 2006 Jun. 9-11; 大阪.	日本

研究報告の概要

○遡及調査からみた残存リスクの評価

HBV、HCV、HIVについて、2000年2月から2004年1月までの4年間の遡及調査と、従来のヘモビジランスの結果から、日本での輸血感染症の実態を報告する。遡及調査は、主に複数回献血者において感染症マーカーが陽転した場合に前回の血液のサンプルを個別NATで精査するもので、HBVについては50プールNAT、HBsAg、HBcAbのいずれかの陽転例約1万6千人について前回の保管検体を調べた。全体で1.01%が個別NATで陽性であった。HBcAbの有無でこれを分類すると、その60%はHBV感染既往者(オカルトキャリアー)、40%がウインドウピリオドのドナーによるものであった。これらの個別NATのみ陽性の血液を輸血された患者63人の輸血前後の情報を集めると、感染が確認された患者は12人のみであった(感染確率19%)。感染を起こした血液をHBcAbの有無で分類すると、ウインドウ期由来の血液は22本のうち11本が感染を起こした(感染確率50%)のに対し、キャリア由来の血液は33本のうち1本のみが感染を起こした(感染確率3%)。前者の感染性は後者の10倍以上高いことになる。感染を起こした製剤と起こさなかった製剤とで、含まれるHBVのコピー数には違いはなかった。赤血球製剤もFFPと同じく高い感染性を有していた。感染を起こした患者が免疫抑制状態にあるという傾向は認められなかった。1年間に頻回献血者の血液の輸血で起こるHBV感染は、キャリア血液によるもの0.7~0.9例、ウインドウ期の血液によるもの7.1~9.7例と推察される。医療機関からの従来の副作用報告によるものをあわせると、日本では輸血によるHBV感染が1年に約19例起こることが予想される。感染性をもつオカルトキャリアによる頻回の献血がこれからの問題となる。輸血によるHCV感染は4年に1例、HIVは2年に1例起こるものと推定される。

報告企業の意見	今後の対応
輸血用血液製剤のHBV、HCV、HIVについて、2000年2月から2004年1月までの4年間の遡及調査と、医療機関からの副作用報告による感染例をあわせると、日本では輸血によるHBV感染が1年に約19例、HCV感染は4年に1例、HIV感染は2年に1例起こるものと推定されるとの報告である。なお、HBV感染のリスクは、以前13~17例と推計していたが、今回の推計は直近のデータ数も含めたものである。	日本赤十字社では、「血液製剤等に係る遡及調査ガイドライン」(平成17年3月10日付薬食発第0310009号)に基づき、輸血感染症の調査を行っている。HBV/HCV/HIV感染に関する新たな知見等について今後も情報の収集に努める。次世代NAT試薬についての評価、検査方法の改良に向けた開発・検討を進める。

使用上の注意記載状況・その他参考事項等

合成血「日赤」
照射合成血「日赤」

血液を介するウイルス、細菌、原虫等の感染
vCJD等の伝播のリスク

(2)

シンポジウム4　輸血感染症の実態とその対策

SY4-1　遡及調査からみた残存リスクの評価

東京都赤十字血液センター副所長
佐竹正博
TEL：03-3406-1211(303)　FAX：03-3406-7892　E-mail：ma-satake@tokyo.bc.jrc.or.jp

HBV，HCV，HIVについて，2000年2月から2004年1月までの4年間の遡及調査と，従来のヘモビジランスの結果から，日本での輸血感染症の実態を報告する．遡及調査は，主に複数回献血者において感染症マーカーが陽転した場合に前回の血液のサンプルを個別NATで精査するもので，HBVについては50プールNAT，HBsAg，HBcAbのいずれかの陽転例約1万6千人について前回の保管検体を調べた．全体で1.01%が個別NATで陽性であった．HBcAbの有無でこれを分類すると，その60%はHBV感染既往者(オカルトキャリアー)，40%がウィンドウピリオドのドナーによるものであった．これらの個別NATのみ陽性の血液を輸血された患者63人の輸血前後の情報を集めると，感染したことが確認された患者は12人のみであった(感染確率19%)．感染を起こした血液をHBcAbの有無で分類すると，ウィンドウ期由来の血液は22本のうち11本が感染を起こした（感染確率50%）のに対し，キャリア由来の血液は33本のうち1本のみが感染を起こした(感染確率3%)．前者の感染性は後者の10倍以上高いことになる．感染を起こした製剤と起こさなかった製剤とで，含まれるHBVのコピー数には違いはなかった．赤血球製剤もFFPと同じく高い感染性を有していた．感染を起こした患者が特に免疫抑制状態にあるという傾向は認められなかった．1年間に頻回献血者の血液の輸血で起こるHBV感染は，キャリア血液によるもの0.7～0.9例，ウィンドウ期の血液によるもの7.1～9.7例と推察される．医療機関からの従来の副作用報告によるものをあわせると，日本では輸血によるHBV感染が1年に約19例起こることが予想される．感染性をもつオカルトキャリアによる頻回の献血がこれからの問題となる．輸血によるHCV感染は4年に1例，HIVは2年に1例起こるものと推定される．

別紙様式第2-1

No. 27

医薬品 研究報告 調査報告書

識別番号・報告回数			報告日	第一報入手日	新医薬品等の区分	機構処理欄
				2006. 6. 20	該当なし	

一般的名称	(製造承認書に記載なし)	研究報告の公表状況		公表国
販売名(企業名)	合成血「日赤」(日本赤十字社) 照射合成血「日赤」(日本赤十字社)		田守昭博, 柴田弘俊, 吉澤浩司. 第54回日本輸血学会総会; 2006 Jun. 9-11; 大阪.	日本

研究報告の概要

○受血者全数調査による実態の把握

【目的】医療機関において輸血後感染症の全数調査を実施し、赤十字血液センターの協力の下で感染症原因の徹底した調査を行い、輸血後感染症の実態を明らかにする。

【対象と方法】厚生労働省肝炎克服事業の一環として多施設共同研究として計画した。平成15年11月より岩手、大阪、愛媛の3医療機関において血液製剤の非輸血症例を対象とした。輸血前と輸血後3ヶ月目の患者血清を保存し輸血後の血清を赤十字血液センターにてHBV-DNA、HCV-RNA、HIV-RNAをNATにて検査した。陽性項目に関しては輸血前血清にてその有無を調査し、輸血後陽転化した症例を明らかにした。

【結果】輸血後3ヶ月での陽性率は各々HBV3.1%(32/1024)、HCV8.7%(87/1000)HIV0%(0/976)であった。陽性者中、輸血前検査にて陰性例(輸血後陽転例)はHCVとHIVでは1例も認められずHBVのみ9例(0.9%)存在した。このうち1例では献血者保存血清中にHBV DNAを検出し遡及調査の対象例でもあり、受血者血清のHBV DNA塩基配列との一致を確認したため輸血後B型肝炎と診断した。他の8例はルックバックにてHBV DNAを検出できず、感染晩期のHBVキャリアに免疫抑制剤や化学療法によるHBVの再活性化が起こったと考えられた。

【結語】全数検査によりHBV、HCV、HIVの輸血による新規感染例と輸血前からの持続感染の鑑別が可能となったHBVによる輸血後の感染が問題であるが、多くはHBVの再増殖と推定された。

報告企業の意見	今後の対応
医療機関において輸血後感染症の全数調査を実施したところ、輸血後陽転例はHBVで9例(0.9%)存在し、1例のみが輸血後B型肝炎と診断され、他の8例は感染晩期のHBVキャリアで再活性化が起こったと考えられたとの報告である。輸血後肝炎ウイルス感染の調査には、HBVキャリアの再活性化など輸血以外の要因について考慮する必要がある。	日本赤十字社では、HBV、HCV、HIVについて20プールでスクリーニングNATを行い、陽性血液を排除している。HBV/HCV/HIV感染に関する新たな知見等について今後も情報の収集に努める。次世代NAT試薬についての評価、検査方法の改良に向けた開発・検討を進める。

使用上の注意記載状況・その他参考事項等

合成血「日赤」
照射合成血「日赤」

血液を介するウイルス、細菌、原虫等の感染
vCJD等の伝播のリスク

MedDRA/J Ver.9.0J

SY4-2 受血者全数調査による実態の把握

大阪市立大学大学院医学研究科肝胆膵病態内科学[1],
大阪府赤十字血液センター大阪府赤十字血液センター[2],
広島大学大学院医歯薬学総合研究科疫学・疾病制御学[3]
田守昭博[1], 柴田弘俊[2], 吉澤浩司[3]
TEL：06-6645-3811 FAX：06-6645-3813 E-mail：atamori@med.osaka-cu.ac.jp

【目的】医療機関において輸血後感染症の全数検査の実施し，赤十字血液センターの協力の下で感染症原因の徹底した調査を行い，輸血後感染症の実態を明らかにする.【対象と方法】厚生労働省肝炎克服事業の一環として多施設共同研究として計画した．平成15年11月より岩手，大阪，愛媛の3医療機関において血液製剤の被輸血症例を対象とした．輸血前と輸血後3ヶ月目の患者血清を保存し輸血後の血清を赤十字血液センターにてHBV-DNA，HCV-RNA，HIV-RNAをNATにて検査した．陽性項目に関しては輸血前血清にてその有無を調査し，輸血後陽転化した症例を明らかにした.【結果】輸血後3ヶ月での陽性率は各々HBV 3.1%(32/1024)，HCV 8.7%（87/1000)，HIV 0%（0/976）であった．陽性者中，輸血前検査にて陰性例（輸血後陽転例）はHCVとHIVでは1例も認められずHBVのみ9例（0.9%）存在した．この内1例では献血者保存血清中にHBV DNAを検出し遡及調査の対象例でもあり，受血者血清のHBV DNA塩基配列との一致を確認したため輸血後B型肝炎と診断した．他の8例はルックバックにてHBV DNAを検出できず，感染晩期のHBVキャリアに免疫抑制剤や化学療法によるHBVの再活性化が起こったと考えられた.【結語】全数検査によりHBV，HCV，HIVの輸血による新規感染例と輸血前からの持続感染の鑑別が可能となったHBVによる輸血後の感染が問題であるが，多くはHBVの再増殖と推定された.

医薬品 研究報告 調査報告書

識別番号・報告回数			報告日	第一報入手日	新医薬品等の区分	機構処理欄
				2006. 6. 20	該当なし	

一般的名称	(製造承認書に記載なし)	研究報告の公表状況	柚木久雄. 第54回日本輸血学会総会; 2006 Jun. 9-11; 大阪.	公表国 日本
販売名(企業名)	合成血「日赤」(日本赤十字社) 照射合成血「日赤」(日本赤十字社)			

研究報告の概要

○核酸増幅検査によるウイルス陽性血液の検出状況と陽性献血者のウイルス学的解析

日本赤十字社では、HBV、HCV、HIV-1の3ウイルスに対する核酸増幅検査(Nucleic acid Amplification Testing、NAT)を1999年7月に開始した。その間プールサイズも500から50、2004年8月からは20と下げてきた。2005年12月までにNATで33,735,075例検査しHBV、HCV、HIV陽性数はそれぞれ625、93、12例であった。以下NAT陽性者の86%を占めるHBV NAT陽性者について解析した。年齢別のNAT陽性率は男性では20歳代が最も高く加齢と伴に減少していたが、50歳代、60歳代では逆に増加していた。この原因を解析するためHBV NAT陽性血液のHBc抗体を測定すると、HBV感染初期と考えられるHBc抗体陰性の陽性率は20歳代以降以降加齢とともに減少していた。これに対し、感染晩期と考えられるHBc抗体陽性者は陽性率が40歳代まではほぼゼロであるが、50歳代、60歳代では陽性率が上昇していた。感染晩期の陽性例が増加した結果、高年齢層でHBV陽性率が上昇したものと考えられる。同様の傾向が女性群でも観測された。20プールになって感度が上がりウイルス濃度の低い検体の比率が高まっていたが、HBV陽性率は50プールで1/52000、20プールでは1/53000と差が見られなかった。20プールでは40歳代以降の献血者の比率が高くなっていた。これは2004年10月に全国で献血者の本人確認を実施したため感染初期の例数が減少したが、逆に感度上昇に伴い感染晩期の例数が増加したため全体として陽性率が変らなかったものと考えられる。HBV Genotype Aは101例(女性1例)検出された。陽性数の高い地域は千葉県から愛知県までの太平洋側と大阪府であった。その周辺で2005年新たにGenotype Aを検出した県が6県あり、全国に広がる傾向が見られた。亜型Ae(欧米型)が84%を占め、更に核酸配列の変異が2塩基以内の株がそのうち74%あり感染源が近い可能性を示唆していた。

報告企業の意見	今後の対応
日本の献血者におけるHBV NAT陽性者について解析したところ、20歳代ではHBV感染初期、50歳代、60歳代では感染晩期の陽性例が多かった。HBV Genotype Aは101例(女性1例)検出され、全国に広がる傾向が見られたとの報告である。	日本赤十字社では、HBV、HCV、HIVについて20プールでスクリーニングNATを行い、陽性血液を排除している。HBV/HCV/HIV感染に関する新たな知見等について今後も情報の収集に努める。次世代NAT試薬についての評価、検査方法の改良に向けた開発・検討を進める。

使用上の注意記載状況・その他参考事項等

合成血「日赤」
照射合成血「日赤」

血液を介するウイルス、細菌、原虫等の感染
vCJD等の伝播のリスク

日本輸血学会雑誌　第52巻　第2号

SY4-5　核酸増幅検査によるウイルス陽性血液の検出状況と陽性献血者のウイルス学的解析

日本赤十字社中央血液研究所核酸増幅検査部
柚木久雄
TEL：03-5713-8837　FAX：03-5713-8838　E-mail：yugi@bs.jrc.or.jp

日本赤十字社ではHBV, HCV, HIV-1の3ウイルスに対する核酸増幅検査(Nucleic acid Amplification Testing以下NATと略）を1999年7月に開始した．その間プールサイズも500から50, 2004年8月からは20と下げてきた．2005年12月までにNATで33,735,075例検査しHBV, HCV, HIV陽性数はそれぞれ625, 93, 12例であった．以下NAT陽性者の86%を占めるHBV DNA陽性者について解析した．年齢別のNAT陽性率は男性では20歳代が最も高く加齢と伴に減少していたが，50歳代，60歳代では逆に増加していた．この原因を解析するためHBV DNA陽性血液のHBc抗体を測定すると，HBV感染初期と考えられるHBc抗体陰性の陽性率は20歳代以降加齢とともに減少していた．これに対し，感染晩期と考えられるHBc抗体陽性者は，陽性率が40代まではほぼゼロであるが，50歳代，60歳代では陽性率が上昇していた．感染晩期の陽性例が増加した結果，高年齢層でHBV陽性率が上昇したものと考えられる．同様の傾向が女性群でも観測された．20プールになって感度が上がりウイルス濃度の低い検体の比率が高まっていたが，HBV陽性率は50プールで1/52000, 20プールでは1/53000と差が見られなかった．20プールでは40歳代以降の献血者の比率が高くなっていた．これは2004年10月に全国で献血者の本人確認を実施したため感染初期の例数が減少したが，逆に感度上昇に伴い感染晩期の例数が増加したため全体として陽性率が変らなかったものと考えられる．HBV Genotype Aは101例（女性1例）検出された．陽性数の高い地域は千葉県から愛知県までの太平洋側と大阪府であった．その周辺で2005年新たにGenotype Aを検出した県が6県あり，全国に広がる傾向が見られた．亜型Ae（欧米型）が84%を占め，更に核酸配列の変異が2塩基以内の株がそのうち74%あり感染源が近い可能性を示唆していた．

別紙様式第2-1

No. 29

医薬品 研究報告 調査報告書

識別番号・報告回数			報告日	第一報入手日	新医薬品等の区分	機構処理欄
				2006. 6. 20	該当なし	

一般的名称	(製造承認書に記載なし)	研究報告の公表状況	百瀬俊也, 平力造, 沼本高志, 伊藤綾香, 後藤直子, 日野学, 柚木久雄, 金光公浩, 田所憲治. 第54回日本輸血学会総会; 2006 Jun. 9-11; 大阪.	公表国
販売名(企業名)	合成血「日赤」(日本赤十字社) 照射合成血「日赤」(日本赤十字社)			日本

研究報告の概要

○医療機関から寄せられた輸血感染症報告の現状(2005年)

2005年に全国の医療機関から日赤血液センターへ報告された輸血感染症(疑い症例を含む)の現状とその解析結果について報告する。

【対象と方法】医療機関から輸血による感染を疑われて報告された症例を対象とした。ウイルス感染(疑い)症例については当該献血者の保管検体の個別NAT等により、また細菌感染(疑い)症例については当該製剤又は同一製造番号の凍結血漿の無菌試験等により、調査を行い評価した。

【結果と考察】2005年輸血感染症(疑い)症例の報告数は260例(12月22日現在)であり、前年の293例に比べ減少した。その内訳はHBV:127、HCV:71、HEV:2、HIV:2、CMV:3、ヒトパルボウイルスB19(以下B19):3、細菌:52であった。このうち輸血との因果関係が高いと評価した症例はHBV:10例、HCV:1例、HEV:1例、B19:3例であった。HBV10例の情報入手別内訳は、自発報告5例、遡及調査4例、追跡調査1例であった。これにはHBV低濃度既往感染者の献血による感染症例と思われる症例が2例、個別NATウインドウ期と考えられる症例が3例含まれていた。HCVは、50プールNAT導入後輸血による感染が確認できた初めての症例である。HEVは、北海道地域で研究的に実施しているHEV-NATにより明らかになった症例である。B19の3例については、調査したNS1/VP1領域1069bpの塩基配列が献血者検体と受血者献体とですべて一致した。遡及調査および感染症報告の解析を基礎としたHBVの過去に公表している感染リスクの推計値(年間13～17例)と比較し、過去3年間のHBV感染症例(13+20+10例)の平均値は14例とこれを裏付けた数値となった。今後の安全対策のさらなる向上とともに、医療機関では国の遡及調査ガイドラインに示されたように「指針」に基づく適正使用の推進と輸血前後検査の確実な実施が望まれる。

報告企業の意見	今後の対応
2005年に全国の医療機関から日本赤十字社へ報告された輸血後感染症(疑い症例を含む)の現況とその傾向についての報告である。	日本赤十字社では、「血液製剤等に係る遡及調査ガイドライン」(平成17年3月10日付薬食発第0310009号)に基づき、輸血感染症の調査を行っている。今後も引き続き、感染症自発報告と遡及調査をあわせて情報の収集に努める。

使用上の注意記載状況・その他参考事項等

合成血「日赤」
照射合成血「日赤」

血液を介するウイルス、細菌、原虫等の感染
vCJD等の伝播のリスク

O-16 医療機関から寄せられた輸血感染症報告の現状（2005年）

日本赤十字社血液事業本部
百瀬俊也，平 力造，沼本高志，伊藤綾香，後藤直子，日野 学，柚木久雄，金光公浩，田所憲治
TEL：03-5733-8223 FAX：03-5402-7627 E-mail：momose@bs.jrc.or.jp

【はじめに】日本赤十字社（以下，日赤）では，薬事法に基づき副作用・感染症症例を独立行政法人医薬品医療機器総合機構へ報告している．2005年に全国の医療機関から日赤血液センターへ報告された輸血感染症（疑い症例を含む）の現状とその解析結果について報告する．
【対象と方法】医療機関から輸血による感染を疑われて報告された症例を対象とした．ウイルス感染（疑い）症例については当該献血者の保管検体の個別NAT等により，また細菌感染（疑い）症例については，当該製剤又は同一製造番号の凍結血漿の無菌試験等により，調査を行い評価した．
【結果と考察】2005年輸血感染症（疑い）症例の報告数は260例（12月22日現在）であり，前年の293例に比べ減少した．その内訳はHBV：127，HCV：71，HEV：2，HIV：2，CMV：3，ヒトパルボウイルスB19（以下，B19）：3，細菌：52であった．このうち輸血との因果関係が高いと評価した症例は，HBV：10例，HCV：1例，HEV：1例，B19：3例であった．
HBV10例の情報入手別内訳は，自発報告5例，遡及調査4例，追跡調査1例であった．この中にはHBV低濃度既往感染者の献血による感染症例と思われる症例が2例，個別NATウインドウ期と考えられる症例が3例含まれていた．HCVについては，50プールNAT導入後輸血による感染が確認できた初めての症例である．HEVについては，北海道地域で研究的に実施しているHEV-NATにより明らかになった症例である．B19の3例については，調査したNS1/VP1領域1069bpの塩基配列が献血者検体と受血者検体とですべて一致した．
遡及調査および感染症報告の解析を基礎としたHBVの感染リスクの推計値（年間13～17例）と比較し，過去3年間のHBV感染症例（13＋20＋10例）の平均値は14例とこれを裏付けた数値となった．今後の安全対策の更なる向上とともに，医療機関では国の遡及調査ガイドラインに示されたように「指針」に基づく適正使用の推進と輸血前後検査の確実な実施が望まれる．

別紙様式第2-1

No. 30

医薬品 研究報告 調査報告書

識別番号・報告回数			報告日	第一報入手日	新医薬品等の区分	機構処理欄
				2006. 6. 20	該当なし	

一般的名称	(製造承認書に記載なし)	研究報告の公表状況	E. Martinez-Bauer, X. Forns, M. Armelles, R. Planas, R. Sola, M. Vergara, S. Fabregas, R. Vega, J. Salmeron, M. Buti, J.M. Sánchez-Tapias, M. Bruguera. 41st Annual Meeting of the European Association for the Study of the Liver; 2006 Apr. 26-30; Vienna.	公表国
販売名(企業名)	合成血「日赤」(日本赤十字社) 照射合成血「日赤」(日本赤十字社)			スペイン

研究報告の概要

○入院は大部分の急性C型肝炎症例における唯一の疫学的リスクファクターである

疫学的研究により、西側諸国においては薬剤の静脈内投与が急性C型肝炎に関連する主なリスクファクターであることが示された。しかし、医療行為に関連する処置により伝播される急性C型肝炎の孤発例は、集団発生と同様、院内感染によるC型肝炎への注目を集めている。この理由により、スペインの肝臓及び消化器専門クリニック数施設を調査し、急性C型肝炎の全報告例について後方視的疫学解析を行った。1998年から2005年の間、急性C型肝炎103例の記録がまとめられた。55例(53%)が男性で感染者の年齢の中央値は46歳であった(21-87歳)。診断時のトランスアミナーゼの中央値は、ASTが471IU/L、ALTが942IU/Lであった。診断前6ヵ月間のリスクファクターの内訳は、入院67例(65%)、麻薬の静脈内投与10例(10%)、不特定多数との性交渉8例(8%)、針刺し事故6例(6%)、歯科受診4例(4%)、血液透析1例(1%)、鍼治療1例(1%)、コカイン吸入1例(1%)の他、明らかなリスクファクターを認めなかったものが5例あった。入院のみがリスクファクターと考えられた67例のうち30例は外科手術、16例は診断的検査を受けており、9例はバイアルの複数回使用による集団発生の症例であった。45例は治療の適応とはならなかった(21例はHCV感染の自然治癒のため、その他は禁忌であったため)。よって、58例が抗ウイルス療法を受け、49例(84%)が持続的なウイルス学的反応に至った。要約すると、急性C型肝炎患者の大部分では、感染に関連する唯一の立証されたリスクファクターは入院である(院内感染であると指摘)。本研究により、統一的な予防手段を厳格に守ることの必要性が強調された。幸い、大部分の急性C型肝炎は、自然に、又は抗ウイルス療法により治癒した。

報告企業の意見	今後の対応
スペインの肝臓及び消化器専門クリニック数施設における急性C型肝炎の全報告例について後方視的疫学解析を行ったところ、大部分の症例で感染に関連する唯一の立証されたリスクファクターは入院であったとの報告である。輸血後HCV感染症の調査には、院内感染など輸血以外の伝播ルートについて考慮する必要がある。	HCV感染の新たな伝播ルート等について、今後も情報の収集に努める。

使用上の注意記載状況・その他参考事項等

合成血「日赤」
照射合成血「日赤」

血液を介するウイルス、細菌、原虫等の感染
vCJD等の伝播のリスク

540

HOSPITAL ADMISSION IS THE ONLY EPIDEMIOLOGICAL RISK FACTOR IN MOST CASES OF ACUTE HEPATITIS C

E. Martinez-Bauer[1], X. Forns[1], M. Armelles[2], R. Planas[3], R. Sola[4], M. Vergara[5], S. Fabregas[6], R. Vega[6], J. Salmeron[7], M. Buti[8], J.M. Sánchez-Tapias[1], M. Bruguera[1]

[1]Hospital Clinic, Barcelona [2]Departament de Salut, Generalitat de Catalunya [3]Hospital Germans Trias i Pujol, Badalona [4]Hospital del Mar, Barcelona [5]Corporació Parc Taulí, Sabadell [6]Hospital de Figueres [7]Hospital Universitario San Cecilio, Granada [8]Hostpial Vall d'Hebron, Barcelona, Spain

Epidemiological studies have shown that intravenous drug use is the main risk factor associated with acute hepatitis C in the Western World. However, isolated cases of acute hepatitis C, as well as hepatitis C outbreaks, transmitted by health-care related procedures have increased the attention on nosocomial hepatitis C. For this reason, we performed a retrospective epidemiological analysis of all reported cases of acute hepatitis C referred to several Spanish Hepatology and Gastroenterology clinics. Between 1998 and 2005, 103 cases of acute hepatitis C were documented. Fifty-five (53%) occurred in men; the median age of the infected individuals was 46 years (21-87). At the time of diagnosis the median AST and ALT values were 471 IU/L and 942 IU/L, respectively. Risk factors registered during the 6-month period preceding the diagnosis of acute hepatitis C were: hospital admission 67 (65%), intravenous drug use 10 (10%), promiscuous sex 8 (8%), accidental needle stick 6 (6%), dentist visit 4 (4%), hemodialysis 1 (1%), acupuncture 1 (1%), cocaine inhalation 1 (1%) and no apparent risk factors 5 (5%). Among the 67 patients in whom hospital admission was the only risk factor, 30 underwent surgery, 16 a diagnostic test and 9 were part of an outbreak linked to the use of a multidose vial. In 45 patients treatment was not indicated (in 21 due to spontaneous resolution of HCV infection and in the remaining due to contraindication). Therefore, 58 patients underwent antiviral therapy and 49 (84%) achieved a sustained virological response. In summary, in most patients with acute hepatitis C the only documented risk factor associated with the infection is a hospital admission (pointing out to its nosocomial origin). The results of this study stress the need of strict adherence to universal precaution measures. Fortunately, most cases of acute hepatitis C resolve spontaneously or after antiviral therapy.

医薬品
医薬部外品　研究報告　調査報告書
化粧品

別紙 3-6

識別番号・報告回数		回	報告日 年　月　日	第一報入手日 2006 年 4 月 6 日	新医薬品等の区分 該当なし	総合機構処理欄
一般的名称			研究報告の公表状況	Correlates of hepatitis C virus (HCV) RNA negativity among HCV-seropositive blood donors Busch, M.P. et al, Transfusion. 46, 469 - 475 (2006)	公表国 米国	
販売名（企業名）						

研究報告の概要	使用上の注意記載状況・その他参考事項等
C 型肝炎ウイルス (以下, HCV) 感染した約 20%の人はウイルス血症が消滅するが, 理由は明確ではない。一方, HCV 初感染者の大多数は無症候であるが, 数十年後に臨床症状を発症する。すなわちこれは, 供血者の中に臨床上の潜在 HCV 感染者がいるということである。 本研究では, 1999 年～2001 年 12 月に NAT を実施した合衆国の 5 つの血液センターで集められた 257 万 9290 名の同種供血者における HCV 抗体と核酸増幅検査 (以下, NAT) データ, 肝酵素アラニン・アミノトランスフェラーゼ (ALT) を調査すると同時に人口学的特性 (年齢, 性別, 人種及び教育レベル) も調査した。 NAT による HCV RNA 陰性患者は, セロコンバージョンまでの期間にかかわらず, 初回供血者 (19.6%) より反復供血者 (55.9%) の方が多かった。初回供血の HCV RNA 陰性者と RNA 陽性者の ALT レベルを比較すると, ALT が 60IU/L 未満の患者の比率はそれぞれ 98.51%, 83.78%, ALT が 120IU/L 以上ではそれぞれ 0.75%, 13.38%であり, ALT レベルは初回供血の HCV RNA 陰性者の方がより正常に近い傾向を示した。 また, 初回供血者の HCV RNA 陰性の比率は, アジア系, 非ヒスパニック黒人系よりも (非ヒスパニック) 白人系で有意に高かった。 さらに有意ではないが, 男性より女性のほうが NAT 陰性の比率が高かった。 なお, この調査は, HCV 罹患率・有病率の高い合衆国南東部を除いた血液センターで実施されており, NAT は個々の供血者ではなくミニプールに対して行われた。	BYL-2006-0214

報告企業の意見	今後の対応
弊社の血漿分画製剤においても, 1988 年よりミニプール及びプール血漿での HCV に対する NAT を実施しているが, 無症候セロコンバージョンが起きた供血者の血漿が使用される可能性は否定できない。しかしながら, 製造工程におけるウイルス除去工程により, 血漿分画製剤の HCV に関する安全性は確保されていると考えられる。	現時点で新たな安全対策上の措置を講じる必要は無いと考える。引き続き関連情報の収集に努める。

TRANSFUSION COMPLICATIONS

Correlates of hepatitis C virus (HCV) RNA negativity among HCV-seropositive blood donors

Michael P. Busch, Simone A. Glynn, Susan L. Stramer, Jennie Orland, Edward L. Murphy, David J. Wright, and Steven Kleinman for the NHLBI Retrovirus Epidemiology Donor Study (REDS) Group

BACKGROUND: Approximately 20 percent of persons infected with hepatitis C virus (HCV) clear viremia. Factors associated with resolution of viremia are not well defined. Implementation of routine nucleic acid testing (NAT) of blood donors has yielded a large data set for analysis of demographic correlates of resolved viremia.

STUDY DESIGN AND METHODS: HCV antibody and NAT data, liver enzyme (alanine aminotransferase [ALT]) results, and donor demographic characteristics were compiled for 2,579,290 allogeneic donations given at five large blood centers after NAT implementation in 1999 through December 2001. Donation HCV RNA status was compared between first-time donors categorized by ALT levels, sex, age, race and/or ethnicity, country of birth, level of education, blood center location, and blood group, with chi-square tests and multivariable logistic regression methods.

RESULTS: Of 35 confirmed-seropositive repeat donors, 19 (54.3%) tested negative for the presence of HCV RNA; there was no association between RNA status and preseroconversion intervals ($p = 0.74$). Of 2105 RIBA-positive, first-time donors, 402 (19.1%) tested negative for the presence of HCV RNA by NAT (presumptive resolved infections). There were significant differences in the frequency of RNA negativity among first-time donors categorized by ALT levels and by race and/or ethnicity. ALT levels were more likely to be elevated in RNA-positive, first-time donors ($p < 0.0001$). Viremia was less likely to resolve in Asian (8.2%) and black non-Hispanic (14.4%) donors than in white non-Hispanic (20.7%), Hispanic (22.1%), and other race and/or ethnicity (22.1%) donors ($p = 0.02$). No significant associations were found for age, sex, country of origin, level of education, blood type, and donor center location.

CONCLUSION: These results confirm that the frequency of HCV RNA negativity among seropositive persons differs by race and/or ethnicity. Follow-up studies of donors with resolved viremia are warranted to further elucidate viral, immunologic, and genetic factors underlying spontaneous viral clearance.

Only about 15 percent of persons recently infected with hepatitis C virus (HCV) develop a clinically overt hepatitis syndrome, which is generally mild, occurs within 5 to 12 weeks of exposure (mean, 8 weeks), and lasts from 2 to 12 weeks.[1,2] Although 85 percent of primary HCV infections are asymptomatic, most studies indicate that 70 to 80 percent of these infections become chronic with asymptomatic viremia generally persisting for decades before later disease manifestations.[1,3] This results in a reservoir of clinically occult HCV infections in persons presenting to donate blood.[3,4] HCV seroprevalence (confirmed by

ABBREVIATIONS: ARC = American Red Cross; BCP = Blood Centers of the Pacific; IDU(s) = injection drug use(rs); MP(s) = minipool(s); REDS = Retrovirus Epidemiology Donor Study; TMA = transcription-mediated amplification.

From the Blood Systems Research Institute, San Francisco, California; the Department of Laboratory Medicine and the Department of Medicine, University of California, San Francisco, San Francisco, California; Westat, Inc., Rockville, Maryland; the American Red Cross, Scientific Support Office, National Testing and Reference Laboratories and Biomedical Services, Gaithersburg, Maryland; and the Department of Pathology, University of British Columbia, Victoria, British Columbia, Canada.

Address reprint requests to: Michael P. Busch, MD, PhD, Vice President, Research and Scientific Affairs, Blood Centers of the Pacific, 270 Masonic Avenue, San Francisco, CA 94118; e-mail: mpbusch@itsa.ucsf.edu.

This work was supported by the National Heart, Lung and Blood Institute's (NHLBI) Retrovirus Epidemiology Donor Study (REDS), Contracts N01-HB-97077 (superseded by N01-HB-47114), -97078, -97079, -97080, -97081, -97082, -47174, and -58181 and by a grant from NHLBI R01-HL-076902.

Received for publication May 5, 2005; revision received July 14, 2005, and accepted July 17, 2005.

doi: 10.1111/j.1537-2995.2006.00745.x

TRANSFUSION 2006;46:469-475.

recombinant immunoblot assay [RIBA]) among first-time donors in the United States ranges from 0.2 to 0.3 percent (reflecting a steady decrease from 0.6% in 1992 owing to increasingly effective education and deferral procedures). With approximately 2.6 million first-time donations per year in the United States, we estimated that 6000 to 9000 HCV-seropositive infections are diagnosed annually as a result of donor screening (data obtained from the American Red Cross [ARC] national database). Since introduction of nucleic acid amplification testing (NAT) in 1999, the viremia status of seropositive donors is routinely available. In addition, NAT screening detects approximately 60 additional HCV-infected donors in the acute viremic, seronegative phase of infection each year (data obtained from the ARC national database).[5,6] Results from interview studies of seropositive US blood donors have demonstrated that injection drug use (IDU) many years before the HCV-positive donation is the likely source of infection for the majority of cases, with transfusions before implementation of HCV screening and other parenteral and sexual risk factors implicated to a lesser extent.[7,8]

Evidence from human and chimpanzee studies indicates that long-term viremia status (i.e., clearance vs. persistence) is generally determined within 6 months but occasionally as long as 12 to 24 months after infection.[1,9-11] The mechanisms and determinants of apparent viral clearance after seroconversion (as demonstrated by RNA negativity but HCV seropositivity) are not well understood but likely involve a combination of host and viral factors, including mode of acquisition; dose and quasispecies complexity of HCV in the source inoculum; viral genotype and/or subtype; patient race and/or ethnicity, HLA type, sex, and age at infection; and numerous host immune response variables.[12-21] Of note, symptomatic acute infections have been associated with an increased rate of viral clearance (up to 50%) compared to asymptomatic infections, probably reflecting the dual effect of a more robust immune response that eradicates infected hepatocytes and results in manifestations of clinical hepatitis.[1,2]

Owing to the large numbers, diversity, and low-risk characteristics of HCV-infected blood donors, this population may offer unique insights into correlates of HCV resolution, relative to those from studies of patients with symptomatic acute infections, historically identified transfusion recipients, or prospectively followed high-risk populations (primarily active IDUs).[1,3] To investigate laboratory and demographic correlates of HCV RNA negativity in HCV-seropositive blood donors, we compiled data on first-time allogeneic (whole-blood community, directed, and apheresis) donations collected at five blood centers participating in the National Heart, Lung, and Blood Institute Retrovirus Epidemiology Donor Study (REDS) that tested HCV-seropositive between the start of HCV RNA NAT in mid-1999 through December 31, 2001. We also evaluated the NAT status of repeat allogeneic donors who gave an HCV antibody–positive donation in the NAT period.

MATERIALS AND METHODS

Five blood centers collecting approximately 8 percent of all US allogeneic donations participated in REDS including three ARC Blood Services regions (Greater Chesapeake and Potomac Region [Baltimore, MD, and Washington, DC], Southeastern Michigan Region [Detroit, MI], and Southern California Region [Los Angeles, CA]); the Blood Centers of the Pacific (BCP, San Francisco, CA); and the Sylvan N. Goldman Oklahoma Blood Institute ([Oklahoma City, OK]). Information on donation type, donation date, demographic characteristics (age, sex, race and/or ethnicity, education level, country of birth), first-time and repeat donor status, and screening and confirmatory test results have been compiled in a database by the REDS coordinating center (Westat, Inc., Rockville, MD) since 1991. The REDS protocol was approved by the institutional review board at each center.

We identified first-time allogeneic (whole-blood community or directed and apheresis) donations that were confirmed HCV antibody–positive and were collected between April 1999, the approximate start of HCV NAT screening at REDS centers, and December 31, 2001 (the study period). The data set for the three ARC centers was subsequently limited to HCV-seropositive donations given subsequent to September 8, 1999, because before that date seroreactive donations were frequently identified before pooling and consequently not subjected to NAT screening. During the study period, screening for antibodies to HCV was conducted with a third generation enzyme-linked immunosorbent assay (Ortho HCV Version 3.0 ELISA test system, Ortho-Clinical Diagnostics, Inc., Raritan NJ). A third-generation (Chiron RIBA HCV 3.0 strip immunoblot assay, Chiron Corp., Emeryville, CA) was used as the confirmatory test by all centers, except for one center (BCP), which used either second-generation RIBA HCV or third-generation HCV assay versions between April and July 1999, before switching exclusively to the third-generation version on July 15, 1999. NAT was conducted with a human immunodeficiency virus-1 (HIV-1) and HCV transcription-mediated amplification (TMA) system (Procleix, Gen-Probe, Inc., San Diego, CA) at four centers (ARC centers and BCP) and a second-generation HCV screening test (COBAS AmpliScreen, Roche Molecular Systems, Inc., Pleasanton CA) at one center (Oklahoma Blood Institute).[6,22] For the Procleix TMA system, minipools (MPs) consisting of 16 individual donations were screened by the multiplex HIV-1 and HCV assay (except for BCP where donations were screened with MPs of 24 for the initial 14 months of NAT screening).[6,22,23] Once a reactive MP was identified, the corresponding individual donations were tested by the multiplex HIV-1 and HCV

assay, followed, if reactive, by discriminatory HCV and HIV-1 TMA assays to identify the viremic donation. For this analysis, only those specimens that tested reactive by the discriminatory HCV TMA were categorized as HCV RNA–positive. The COBAS AmpliScreen HCV assay was performed on MPs of 24 donations, with two-step resolution with six-member intermediate pools, followed by individual donation testing to identify the HCV RNA–positive donation(s). Both the Procleix and the AmpliScreen systems are highly sensitive with 50 percent detection limits of 12 HCV copies per mL on neat samples based on probit analyses and projected sensitivities of MP NAT screening of fewer than 250 copies per mL.[22,23]

We also identified repeat allogeneic donors who had given at least one third-generation HCV ELISA–nonreactive donation since January through June 1996 (time of third-generation HCV ELISA implementation) followed by an HCV ELISA–reactive and third-generation RIBA–positive donation in the NAT study period (April-September 1999, to December 31, 2001). We evaluated the proportions of third-generation RIBA–positive donations that were RNA-positive or -negative as a function of estimated time since infection by evaluating NAT status as a function of the length of the seroconversion interval or time between the HCV antibody–negative and HCV antibody–positive donations.

To evaluate if HCV RNA negativity in seropositive donors was associated with demographic characteristics, we evaluated the number of HCV RNA-negative (presumptive resolved infections) and RNA-positive (presumptive chronic infections) donations from first-time seropositive donors in various demographic groups and used chi-square tests to compare the proportions of HCV RNA-negative donations between demographic groups. The demographic characteristics evaluated included age (<25, 25-34, 35-44, 45-54, 55-65, and ≥65 years old), sex, race and/or ethnicity (white non-Hispanic, black non-Hispanic, Hispanic, Asian, other non-Hispanic), country of birth (US; non-US), education level (less than high school degree, high school degree, some college or an associate degree, college degree, graduate or professional degree), and blood center location. We also assessed if the distribution of ABO/Rh blood groups and alanine aminotransferase (ALT) levels (normal, <60 IU/L; low-elevated, 60-120 IU/L; high-elevated, >120 IU/L) differed between the RNA-negative and RNA-positive groups. Missing data were excluded from the analysis (NAT results were not available for 2.4% of donations from HCV antibody–positive, first-time donors; race and/or ethnicity, level of education, and country of birth information were missing for 11.3, 9.3, and 3.8 percent of donations from RIBA-positive first-time donors, respectively; and information on age, sex, blood center, ABO/Rh, and ALT was missing for 0.1% of donations from first-time donors). Logistic regression models were conducted to obtain odds ratios (ORs) that compared the odds of being HCV RNA-negative between demographic groups. We included as independent variables in the adjusted models only those demographic characteristics with a chi-square p value of 0.1. The models were conducted both unadjusted (one demographic characteristic at a time) and adjusted (all demographic variables entered into the model). The adjusted model permitted us to evaluate whether the association between a particular demographic variable and NAT status was confounded by another demographic variable, that is, whether the apparent effect of one demographic variable on NAT nonreactivity could be attributed to the effect of another demographic variable on NAT status.

RESULTS

The five participating blood centers collected 2,579,290 allogeneic donations during the study period (start of routine MP-NAT of seroreactive donations in 1999 through December 31, 2001), including 616,228 first-time (23.9%) and 1,963,062 (76.1%) repeat donations. There were 2105 donations from HCV ELISA–reactive and RIBA-positive first-time donors (seroprevalence, 0.34%); of these 402 (19.6%) tested nonreactive and 1653 (78.5%) reactive by MP-NAT (50 donations [2.4%] from RIBA-positive, first-time donors had missing NAT results and were subsequently excluded from the analysis; Table 1). HCV seroprevalence rates were similar among the five REDS centers; the number of confirmed seropositive donors contributed per center varied from 227 (ARC Southeastern Michigan Region) to 729 (ARC Southern California Region).

We also identified 35 HCV seroconverters who gave at least one HCV ELISA–nonreactive donation subsequent to implementation of the third-generation version of the anti-HCV screening assay in 1996, which was followed by an ELISA-reactive and RIBA-positive donation during the current study period. Of these 35 repeat donors, 19 (54.3%) were HCV MP-NAT–nonreactive on their RIBA-positive donation (MP-NAT was not available for 1 positive donation [2.9%]). The median seroconversion interval length observed for the 34 seroconverters with a NAT result was 22.6 months (range, 3.4 to 60.4 months); 24 of the 34 seroconverters (70.6%) had an interval of at least 1 year. We hypothesized that repeat donors with shorter seroconversion intervals would have higher RNA positivity rates than donors with longer seroconversion intervals, since on average they should have been infected more recently and consequently had less time to clear the HCV virus. As shown in Table 1, however, there was no significant difference in HCV RNA rates in seropositive donors with shorter or longer seroconversion intervals ($p = 0.74$).

Further analyses of correlates of RNA negativity were limited to the 2055 first-time confirmed-seropositive

donors for whom NAT status was available. Significant associations ($p < 0.05$) were observed between HCV RNA negativity, and ALT levels (Table 2) and race and/or ethnicity (Table 3). ALT levels were more likely to be normal in RNA-negative donors than in RNA-positive donors, and high-elevated ALT levels (≥120 IU/L) were much more frequently observed in RNA-positive donors than RNA-negative donors ($p < 0.0001$; Table 2). Asian and black non-Hispanic donors were less likely to be RNA-negative than Hispanic, white non-Hispanic, or other non-Hispanic first-time donors ($p = 0.02$; Table 3). The odds of HCV RNA negativity were significantly lower in Asian donors (unadjusted OR, 0.34; 95% confidence interval [CI], 0.12-0.96) and in black non-Hispanic donors (unadjusted OR, 0.64; 95% CI, 0.44-0.93) than in white non-Hispanic donors (Table 4). These racial and/or ethnic differences were not explained by age or sex differences between race and/or ethnic groups because unadjusted and adjusted (for age and sex) ORs remained similar (Table 4).

Associations between HCV RNA negativity and sex ($p = 0.10$), age ($p = 0.12$), blood center location ($p = 0.17$), country of origin ($p = 0.64$), education ($p = 0.80$), and ABO/Rh status ($p = 0.72$) were all nonsignificant (Table 3).

TABLE 1. Proportion of RIBA-positive donations by first-time and repeat donors who tested HCV RNA–negative by MP-NAT and relationship of preseroconversion intervals for repeat donors by RNA status

Donor status	Number of RIBA-positive donations	HCV RNA–negative*	p Value
First-time	2055	402 (19.6)	
Repeat: length of preseroconversion interval (months)	34	19 (55.9)	
≤6	4	3 (75.0)	
>6-9	2	2 (100.0)	
>9-12	4	2 (50.00)	
>12-24	9	4 (44.4)	
>24-60	15	8 (53.3)	0.74

* Data are reported as number (%). MP-NAT results were not available for 50 RIBA-positive, first-time donors (2.4%) and for 1 RIBA-positive, repeat donor (2.9%).

TABLE 2. Proportion of first-time seropositive donations who were HCV RNA-negative or -positive (by MP-NAT) with normal, low-elevated, or high-elevated ALT levels

	ALT level (IU/L)			
HCV status	Normal (<60)	Low-elevated (60-120)	High-elevated (≥120)	p Value
RNA-negative	396 (98.51)	3 (0.75)	3 (0.75)	
RNA-positive	1384 (83.78)	47 (2.85)	221 (13.38)	<0.0001

* Data are reported as number (%).

DISCUSSION

Investigations in various populations at high risk for HCV infection have identified correlates of HCV RNA clearance, including host factors such as age, sex, and race and/or ethnicity. The effectiveness of the immune response (which is largely determined by host genetics but influenced by age, health status, and previous antigenic exposures) and the corresponding evolution of the virus during the preseroconversion and early postseroconversion phases of infection are probably the major determinants of successful resolution of HCV viremia.[12-21,24-26]

Thomas and coworkers[18] first reported that race and/or ethnicity is significantly associated with clearance of HCV RNA in a study of seropositive IDU in Baltimore, Maryland. These investigators found that 9.3 percent of 729 seropositive black non-Hispanic IDUs resolved HCV infection, compared to 36 percent of 44 nonblack donors. Although our findings confirm a significant association between HCV RNA negativity in seropositive first-time blood donors and race and/or ethnicity, the differences in frequencies between black non-Hispanic blood donors (14.4%) and nonblack donors (e.g., 20.7% for white non-Hispanic donors) are smaller than seen with the Baltimore IDU cohort. This difference may relate to the high rate of HIV coinfection in the IDU cohort (33.4% of HCV-seropositive IDUs were HIV-seropositive, and HIV coinfection was associated with lower rates of HCV clearance) and to the relatively small number of nonblack subjects in that study compared to our study (44 vs. 1153, respectively). We also found that the odds of Asian first-time seropositive donors being RNA-positive was approximately three times that of white non-Hispanic donors. To elucidate the basis for these race and/or ethnicity differences, investigators have begun to correlate HLA and other immune response polymorphisms with HCV clearance status. Strong associations with several HLA class I and II alleles have been detected.[19,20] Persons who have cleared HCV are also more likely than chronic carriers to be homozygous for specific inhibitory killer immunoglobulin-like receptors (KIR) combinations (KIR2DL3) and to harbor specific polymorphisms related to cytotoxic lymphocyte antigen 4 (CTLA-4), interleukin 10, and CD4+ CD25+ regulatory T-cells (T-reg; Thio and colleagues, submitted for publication). These genetic studies have been hampered by insufficient power owing to limited numbers of persons with resolved HCV infections from various race and/or ethnicity groups. Access to seropositive blood donors who have tested RNA-negative offers a solution to this problem, and we are now contributing specimens from donors with presumptive resolved infection to these studies.

TABLE 3. Proportion of donations from first-time seropositive donors in a demographic group testing HCV RNA (MP-NAT)-negative or -positive

Demographic group	HCV RNA status* Negative	Positive	p Value
Age (years)			
<25	28 (14.89)	160 (85.11)	
25-34	64 (23.70)	206 (76.30)	
35-44	180 (20.93)	680 (79.07)	
45-54	110 (17.89)	505 (82.11)	
55-64	15 (15.63)	81 (84.38)	
≥65	5 (20.83)	19 (79.17)	0.12
Sex			
Male	232 (18.40)	1029 (81.60)	
Female	170 (21.41)	624 (78.59)	0.10
Race and/or ethnicity			
Asian	4 (8.16)	45 (91.84)	
Black non-Hispanic	38 (14.39)	226 (85.61)	
Hispanic	62 (22.06)	219 (77.94)	
Other non-Hispanic	16 (21.05)	60 (78.95)	
White non-Hispanic	239 (20.73)	914 (79.27)	0.02

* Data are reported as number (%).

TABLE 4. OR comparing odds of being HCV-seropositive and RNA (MP-NAT)-negative by demographic characteristic

Demographic characteristic	Unadjusted OR (95% CI)	Adjusted OR* (95% CI)
Age (years)		
<25	1.0	1.0
25-34	1.78 (1.09-2.90)	2.08 (1.20-3.60)
35-44	1.51 (0.98-2.33)	1.75 (1.07-2.87)
45-54	1.24 (0.79-1.95)	1.48 (0.88-2.46)
55-64	1.06 (0.54-2.09)	1.30 (0.62-2.73)
≥65	1.50 (0.52-4.36)	1.98 (0.66-5.96)
Sex		
Male	1.0	1.0
Female	1.21 (0.97-1.51)	1.14 (0.90-1.45)
Race and/or ethnicity		
White non-Hispanic	1.0	1.0
Asian	0.34 (0.12-0.96)	0.36 (0.13-1.01)
Black non-Hispanic	0.64 (0.44-0.93)	0.66 (0.45-0.96)
Hispanic	1.08 (0.79-1.49)	1.08 (0.78-1.48)
Other	1.02 (0.58-1.80)	1.03 (0.58-1.83)

* Adjusted for age, sex, and race and/or ethnicity.

Several groups have reported that HCV clearance rates correlate with sex (more frequent clearance in women than men) and age at time of infection (young donors resolve infection more frequently than older donors). In our analysis, although nonsignificant, the odds for women to clear viremia appeared 21 percent greater than for men ($p > 0.05$), similar to the findings in several other studies (reviewed in Orland et al.[1]). The association with age was not significant, and no clear trend was observed.

Our results confirm that HCV RNA positivity is associated with elevation of ALT in RIBA-positive donors. Sixteen percent of viremic seropositive donors had ALT elevations, including 13 percent with elevations greater than the high donor screening cutoff of 120 IU per L. This indicates that a substantial subset of viremic donors have active liver disease at the time of donation. Because liver disease in HCV infection is intermittent and follow-up studies of HCV-infected persons with normal ALT have demonstrated low rates of progression to clinical disease, viremic donors without ALT elevation at the time of donation also warrant clinical follow-up to evaluate prognosis and available therapeutic options.[27] In other analyses, we have shown that HCV RNA-negative, RIBA-positive donors (first-time and repeat donors) have a distribution of ALT levels that is essentially identical to that of HCV ELISA–nonreactive donors.[6] This supports the conclusion that the large majority of RNA-negative, anti-HCV–positive donors have no residual hepatic inflammation and have likely eradicated HCV infection. In this data set, the frequency of elevated ALT among RIBA-positive, first-time donors who tested RNA-negative was 1.5 percent, which is slightly higher than rates of ALT elevation among all HCV ELISA–nonreactive donors. This likely relates to the possibility that a minority of these donors still have active HCV infection that is undetectable by a single RNA determination performed in a MP format of 16 to 24 donations.

Our findings of a lower frequency of HCV RNA positivity in seroconverting repeat donors than in seropositive first-time donors was unexpected. We had hypothesized that seroconverters, on average, would have been more recently infected than first-time donors, and if sampled within the first 6 months after the identification of their RNA positivity, would have retained higher rates of viremia not having adequate time for clearance. We observed that 56 percent of seroconverters tested RNA-negative on their first antibody-positive donation, in contrast to 20 percent in first-time donors, with no apparent relationship between viral clearance and interdonation intervals. It is unknown whether these donors have resolved infection or represent individuals with transient low-level viremia that may only be detected intermittently. Of the 34 seroconverting donors identified in our study, 24 (71%) had interdonation intervals exceeding 12 months so that these donors would not have been expected to have different HCV RNA-positive frequencies than first-time donors. The lower percentage of viremia in repeat seropositive donors could relate to recent observations in chimps and humans that in early infection, as the immune system tries to control the infection, RNA levels fluctuate and may be intermittently nondetectable by MP NAT despite eventual development of chronic infection.[9,11] With time, either the immune system is able to eradicate the infection or the virus escapes. Also, demographic differences in first-time and repeat donors may explain the different RNA-positive frequencies observed in these two groups.

This study has several limitations. We used routine donor screening MP-NAT results, resolved to the individual donation level for NAT-reactive pools, to classify RIBA-

positive donors as RNA-positive or -negative. Owing to the 16- to 24-fold dilution factor inherent in MP-NAT, we may have failed to detect viremia in a proportion of seropositive donors with chronic infection (whether these donors were first-time or repeat donors or with interdonation intervals exceeding 12 months). The MP-NAT assays employed in donor screening, however, have 50 percent detection limits of approximately 200 gEq to mL, equivalent to that of commercially available quantitative HCV RNA assays that are widely used clinically to define viremia status of seropositive persons. We have retested specimens from MP-NAT-nonreactive, RIBA-positive donors individually and have identified HCV RNA positivity in 2 to 5 percent of cases; results from follow-up studies of such donors indicate that they have normal ALT levels and intermittent low-level viremia detectable when samples are tested without dilution.[28]

Finally, our current analysis was limited to demographic and laboratory data available from routine donor screening on approximately 8 percent of the US blood supply during the study period; the REDS centers do not include centers from the southeastern United States where HCV incidence and prevalence are higher than in the rest of the country (data from the ARC national database). We also did not recall donors to investigate risk factors or probable dates of infection, nor did we perform additional laboratory studies to confirm viremia status or further characterize the donors (e.g., HLA typing, cellular immune responses) and the virus (subtype, quasispecies diversity). We have recently begun a study that involves enrollment and follow-up of seropositive donors with resolved infections and matched control donors with persistent infections, as well as NAT-positive antibody-negative donors who are being followed prospectively through seroconversion to establish their resolution status. This study will hopefully contribute to the understanding of determinants of HCV clearance, as well as yield information to assist in counseling and clinical management of the several thousand HCV-infected donors identified annually by US blood centers.[29]

ACKNOWLEDGMENTS

The Retrovirus Epidemiology Donor Study (REDS) is the responsibility of the following persons:

Blood centers:

American Red Cross Blood Services Greater Chesapeake and Potomac Region: C.C. Nass

American Red Cross Blood Services Southeastern Michigan Region: M. Higgins

American Red Cross Blood Services Southern California Region: G. Garratty, S. Hutching

Blood Centers of the Pacific-Irwin Centers: E.L. Murphy (UCSF), M.P. Busch (BSRI)

Oklahoma Blood Institute: R.O. Gilcher, J.W. Smith

Medical coordinating center:

Westat, Inc.: G.B. Schreiber, S.A. Glynn, M.R. King

National Heart, Lung, and Blood Institute, NIH:

G.J. Nemo

Steering committee chairman:

T.F. Zuck

REFERENCES

1. Orland JR, Wright TL, Cooper S. Acute hepatitis C [review]. Hepatology 2002;33:321-7.
2. Marcellin P. Hepatitis C. the clinical spectrum of the disease [review]. J Hepatol 1999;31(Suppl 1):9-16.
3. Busch MP. Insights into the epidemiology, natural history and pathogenesis of hepatitis C virus infection from studies of infected donors and blood product recipients. Transfus Clin Biol 2001;8:200-6.
4. Dodd RY, Notari EP 4th, Stramer SL. Current prevalence and incidence of infectious disease markers and estimated window-period risk in the American Red Cross blood donor population. Transfusion 2002;42:975-9.
5. Wang B, Schreiber GB, Glynn SA, et al. Does prevalence of transfusion-transmissible viral infections reflect corresponding incidence in United States blood donors? Transfusion 2005;45:1089-96.
6. Stramer SL, Glynn SA, Kleinman SH, et al. Detection of HIV-1 and HCV infections among antibody-negative blood donors by nucleic acid amplification testing. N Engl J Med 2004;351:760-8.
7. Murphy EL, Bryzman SM, Glynn SA, et al. Risk factors for hepatitis C virus infection in United States blood donors. NHLBI Retrovirus Epidemiology Donor Study (REDS). Hepatology 2000;31:756-62.
8. Orton SL, Stramer SL, Dodd RY, Alter MJ. Risk factors for HCV infection among blood donors confirmed to be HCV RNA-positive, anti-HCV non-reactive. Transfusion 2004; 44:275-81.
9. Prince AM, Pawlotsky JM, Soulier A, et al. HCV replication kinetics in chimpanzees with self-limited and chronic infections. J Viral Hepat 2004;11:236-42.
10. Jauncey M, Micallef JM, Gilmour S, et al. Clearance of hepatitis C virus after newly acquired infection in injection drug users. J Infect Dis 2004;190: 1270-4.
11. Cox AL, Netski DM, Mosbruger T, et al. Prospective evaluation of community-acquired acute hepatitis C. Clin Infect Dis 2005;40:951-8.
12. Zavaglia C, Belli LS, Alberti AB, Ideo G, Silini E. Both immunogenetic factors and HCV genotype can influence the outcome of chronic HCV infection. Am J Gastroenterol 1997;92:725-6.
13. Farci P, Shimoda A, Coiana A, et al. The outcome of acute hepatitis C predicted by the evolution of the viral quasispecies. Science 2000;288:339-44.

14. Sheridan I, Pybus OG, Holmes EC, Klenerman P. High-resolution phylogenetic analysis of hepatitis C virus adaptation and its relationship to disease progression. J Virol 2004;78:3447-54.
15. Laskus T, Wilkinson J, Gallegos-Orozco JF, et al. Analysis of hepatitis C virus quasispecies transmission and evolution in patients infected through blood transfusion. Gastroenterology 2004;127:764-76.
16. Mas A, Ulloa E, Bruguera M, et al. Hepatitis C virus population analysis of a single-source nosocomial outbreak reveals an inverse correlation between viral load and quasispecies complexity. J General Virol 2004;85:3619-26.
17. Ray SC, Wang YM, Laeyendecker O, et al. Acute hepatitis C virus structural gene sequences as predictors of persistent viremia: hypervariable region 1 as a decoy. J Virol 1999; 73:2938-46.
18. Thomas DL, Astemborski J, Rai RM, et al. The natural history of hepatitis C virus infection: host, viral, and environmental factors. JAMA 2000;284:450-6.
19. Thio CL, Thomas DL, Goedert JJ, et al. Racial differences in HLA class II associations with hepatitis C virus outcomes. J Infect Dis 2001;184:16-21.
20. Thio CL, Gao X, Goedert JJ, et al. HLA-Cw*04 and hepatitis C virus persistence. J Virol 2002;76:4792-7.
21. Sugimoto K, Stadanlick J, Ikeda F, et al. Influence of ethnicity in the outcome of hepatitis C virus infection and cellular immune response. Hepatology 2003;37:590-9.
22. Grant PR, Busch MP. Nucleic acid amplification technology methods used in blood donor screening [review]. Transfus Med 2002;12:229-42.
23. Giachetti C, Linnen JM, Kolk DP, et al. Highly sensitive multiplex assay for detection of human immunodeficiency virus type 1 and hepatitis C virus RNA. J Clin Microbiol 2002;40:2408-19.
24. Rehermann B, Nascimbeni MO. Immunology of hepatits B virus and hepatitis C virus infection. Nature Rev Immunol 2005;5:215-29.
25. Cooper S, Erickson AL, Adams EJ, et al. Analysis of a successful immune response against hepatitis C virus. Immunity 1999;10:439-49.
26. Diepolder HM, Gerlach JT, Zachoval R. Immunodominant CD4+ T-cell epitope within nonstructural protein 3 in acute hepatitis C virus infection. J Virol 1997;71:6011-9.
27. Strader DB, Wright T, Thomas DL, Seeff LB. Diagnosis, management, and treatment of hepatitis C [published erratum appears in Hepatology 2004;40:269]. Hepatology 2004;39:1147-71.
28. Tobler LH, Murphy EL, Andrews WW, et al. Follow-up study of HCV seropositive blood donors who tested positive or negative for HCV RNA by minipool-NAT screening. Transfusion 2005;45:92A (abstract SP208).
29. Busch MP, Page-Shafer KA. Acute-Phase hapititis C virus infection: Implications for research, diagnosis, and treatment. Clin Infect Dis 2005;40:959-61.

別紙様式第 2-1
番号 12

医薬品
医薬部外品　　研究報告　調査報告書
化粧品

識別番号・報告回数			報告日	第一報入手日 2006 年 9 月 4 日	新医薬品等の区分 該当なし	厚生労働省処理欄

一般的名称	乾燥抗D（Rho）人免疫グロブリン	研究報告の公表状況	肝臓 2006;47(8):384-391	公表国 日本
販売名（企業名）	抗D人免疫グロブリン-Wf（ベネシス）			

研究報告の概要

わが国の E 型肝炎の実態を明らかにする目的で、全国から総数 254 例の E 型肝炎ウイルス感染例を集め、これを解析した。
その結果、以下の知見を得た。
1)HEV は全国に浸透している。
2)感染者の多くは中高年（平均年齢約 50 歳）で、男性に多い(男女比約 3.5 対 1)。
3)我国に土着の HEV の遺伝型は 3 型と 4 型であり、後者は北海道に多い。
4)年齢と肝炎重症度との間に相関がある。
5)遺伝型 3 型に比べて、4 型は顕在化率も重症化率も高い。
6)発症時期は無季節性である。
7)感染経路は、動物由来食感染が約 30%、輸入感染が 8%、輸血感染が 2%、不明が約 60%であった。

使用上の注意記載状況・その他参考事項等

2. 重要な基本的注意
(1)本剤の原材料となる血液については、HBs 抗原、抗 HCV 抗体、抗 HIV-1 抗体、抗 HIV-2 抗体陰性で、かつ ALT(GPT)値でスクリーニングを実施している。更に、プールした試験血漿については、HIV-1、HBV 及び HCV について核酸増幅検査（NAT）を実施し、適合した血漿を本剤の製造に使用しているが、当該 NAT の検出限界以下のウイルスが混入している可能性が常に存在する。本剤は、以上の検査に適合した血漿を原料として、Cohn の低温エタノール分画で得た画分から抗D（Rho）人免疫グロブリンを濃縮・精製した製剤であり、ウイルス除去を目的として、製造工程において濾過膜処理(ナノフィルトレーション)を施しているが、投与に際しては、次の点に十分注意すること。

報告企業の意見	今後の対応
日本におけるE型肝炎ウイルス感染の統計学的･疫学的･ウイルス学的特徴を求めた調査の解析結果報告である。 万一原料血漿にHEVが混入したとしても、EMCをモデルウイルスとしたウイルスバリデーション試験成績から、本剤の製造工程において十分に不活化・除去されると考えている。	本報告は本剤の安全性に影響を与えないと考えるので、特段の措置はとらない。

肝臓 47巻8号 384-391 (2006)

<原 著>

本邦に於けるE型肝炎ウイルス感染の統計学的・疫学的・ウイルス学的特徴：全国集計254例に基づく解析

阿部 敏紀[1] 相川 達也[2] 赤羽 賢浩[3] 新井 雅裕[4] 朝比奈靖浩[5]
新敷 吉成[6] 茶山 一彰[7] 原田 英治[8] 橋本 直明[9] 堀 亜希子[10]
市田 隆文[11] 池田 広記[12] 石川 晶久[13] 伊藤 敬義[14] 姜 貞憲[15]
狩野 吉康[16] 加藤 秀章[17] 加藤 将[18] 川上 万里[19] 北嶋 直人[20]
北村 庸雄[21] 正木 尚彦[22] 松林 圭二[23] 松田 裕之[24] 松井 淳[25]
道堯浩二郎[26] 三原 弘[27] 宮地 克彦[28] 宮川 浩[29] 水尾 仁志[30]
持田 智[31] 森山 光彦[32] 西口 修平[33] 岡田 克夫[34] 齋藤 英胤[35]
佐久川 廣[36] 柴田 実[37] 鈴木 一幸[38] 高橋 和明[4] 山田剛太郎[39]
山本 和秀[40] 山中 太郎[41] 大和 弘明[42] 矢野 公士[43] 三代 俊治[4]*

要旨：極く最近まで殆んど不明状態にあった我国のE型肝炎の実態を明らかにする目的で，我々は全国から総数254例のE型肝炎ウイルス（HEV）感染例を集め，統計学的・疫学的・ウイルス学的特徴を求めてこれを解析した．その結果，[i] HEV感染は北海道から沖縄まで全国津々浦々に浸透していること；[ii] 感染者の多くは中高年（平均年齢約50歳）で，且つ男性優位（男女比約3.5対1）であること；[iii] 我国に土着しているHEVはgenotype 3とgenotype 4であるが，後者は主に北海道に偏在していること；[iv] 年齢と肝炎重症度との間に相関があること；[v] Genotype 3よりはgenotype 4による感染の方が顕性化率も重症化率も高いこと；[vi] 発生時期が無季節性であること；[vii] 集積症例全体の約30%は動物由来食感染，8%は輸入感染，2%は輸血を介する感染に帰せしめ得たものの，過半の症例（約60%）に於いては感染経路が不明のままであること；等の知見を得た．

索引用語： E型肝炎 E型肝炎ウイルス 疫学 日本

1) 一心病院，2) 相川内科病院，3) 甲府市立甲府病院，4) 東芝病院，5) 武蔵野赤十字病院，6) 富山大学三内，7) 広島大学分子病態制御内科，8) 国立療養所東京病院，9) 東京逓信病院，10) 国保中央病院，11) 順天堂大学静岡病院，12) 関西医大三内，13) 日立総合病院，14) 昭和大学二内，15) 手稲渓仁会病院，16) 札幌厚生病院，17) 豊川市民病院，18) 北海道大学二内，19) 鳥取大学二内，20) 市立加西病院，21) 順天堂大学浦安病院，22) 国立国際医療センター，23) 北海道赤十字血液センター，24) 松田内科医院，25) 至聖病院，26) 愛媛大学三内，27) 高岡市民病院，28) 大阪医大二内，29) 帝京大学溝口，30) 勤医協中央病院，31) 埼玉医大消化器肝臓内科，32) 日本大学消化器肝臓内科，33) 兵庫医大肝胆膵科，34) 鳥取県立中央病院，35) 慶應大学消化器内科，36) ハートライフ病院，37) NTT東日本関東病院，38) 岩手医大一内，39) 川崎医大川崎病院，40) 岡山済生会総合病院，41) 板橋中央総合病院，42) 北見赤十字病院，43) 国立病院機構長崎医療センター
*Corresponding author: shunji.mishiro@po.toshiba.co.jp

＜受付日2006年5月10日＞＜採択日2006年6月26日＞

Table 1 Remarkable predominance of male over female, irrespective of severity of the disease.

Gender	Total n = 243	Disease categories			
		Subclinical n = 71	AH[a] n = 135	ASH[a] n = 21	FH[a] n = 16
Female	55(23%)	18(34%)	29(21%)	4(19%)	4(25%)
Male	188(77%)	53(66%)	106(79%)	17(81%)	12(75%)
F/M ratio	1 / 3.4	1 / 2.9	1 / 3.7	1 / 4.3	1 / 3

[a]Abbreviations : AH, acute hepatitis ; ASH, acute severe hepatitis (defined by prolonged prothrombin time, i.e., PT value < 40%) ; FH, fulminant hepatitis.

Table 2 Age of the subjects, possibly influencing clinical manifestations.

Age in yrs	Total n = 242	Disease categories			
		Subclinical n = 70	AH n = 135	ASH n = 21	FH n = 16
Less than 40	63(26%)	38(54%)[a]	21(16%)	3(14%)	1(6%)
40 to 59	105(43%)	20(29%)	70(52%)	11(53%)	4(25%)
60 or more	74(31%)	12(17%)	44(32%)	7(33%)	11(69%)[b]
Mean ± SD	50.1 ± 15.6	42.3 ± 15.9[c]	52.8 ± 14.4	52.8 ± 15.6	58.9 ± 10.1[d]

[a] P < 0.001, 0.003, < 0.001 against "AH", "ASH", "FH" respectively; [b] P = 0.010, < 0.001 against "AH", "Subclinical" (Chi square test). [c] P < 0.001, 0.009, < 0.001 against "AH", "ASH", "FH" (t test) ; [d] P = 0.047 against "AH" (Welch test).

緒　言

我国や西欧諸国は，アジア・アフリカの熱帯亜熱帯地域諸国と異なり，E型肝炎が頻発する地域ではないから，相当数の症例を集積するには時間と手間がかかる故，100例以上の症例を纏めて解析した報告は，我々の知る限り英文であれ和文であれ一報だに存在しない．我々は，約3年の歳月をかけて，共著者の夫々が過去およびリアルタイムに経験した症例の情報と検体を持ち寄り，更にはこれに我国から学会や論文で発表された症例の情報をも追加し，2006年1月末までに総数254例の，国内で経験されたHEVヒト感染例を集積することを得た．かほどの多数例を纏めて解析した仕事は未見であるし，聊か興味深い知見も得られたので，以下にそれを報告する．

方　法

症例の任意登録

共著者の夫々が，過去及び現在進行形で経験したHEV感染例について，地域，年齢，性，発病年，発病月，病型診断(Subclinical, Acute Hepatitis, Acute Severe Hepatitis, Fulminant Hepatitis のいずれか)，経過中最高ALT値，経過中最高総ビリルビン値，経過中最延長プロトロンビン時間値，ウイルス学的診断根拠（HEV RNA陽性，あるいはIgM抗体・IgG抗体共陽性），HEV genotype，推定あるいは確定された感染経路，海外渡航歴の有無，等の情報を任意登録した．2006年1月末の時点で，この『任意登録』によって集積し得た症例数はn＝206である．尚，HEV RNAが陽性でありながらgenotypingが未施行であった症例については，可能な限り検体の入手に努力し，sequencingを行った（方法後出）．

既報告例の引用登録

国内学会での過去の報告例については，抄録から上記調査項目に相当するデータを拾い集めた．論文発表例[1)-16)]については，一部は，当該論文著者自身から上記調査項目に相当するデータを任意登録して貰ったが，それが不可能であった場合には論文中の記載から該当データを引用登録した．この『引用登録』によって集積し得た症例数はn＝48であり，そのうち最古の症例

Table 3 Geographical distribution of HEV genotypes, showing a significant predominance of type-3 over type-4 in the areas other than Hokkaido.

Areas[a]	Total n = 228	HEV genotype 1	2	3	4
Hokkaido	123	—	—	58(47%)	65(53%)[b]
Tohoku	18	—	—	17(94%)	1(6%)
Kanto-Koshin'etsu	48	5(10%)[c]	—	31(65%)	12(25%)
Chuhbu-Hokuriku	8	1(9%)[c]	—	7(91%)	—
Kinki	10	1(10%)[c]	—	9(90%)	—
Chuh-Shikoku	10	1(10%)[c]	—	7(70%)	2(20%)
Kyushu-Okinawa	11	—	—	9(82%)	2(18%)

[a] Japan was divided, from northeast to southwest, into 7 areas, each of which includes the following prefectures. "Hokkaido" : Hokkaido alone. "Tohoku" : Aomori, Iwate, Miyagi, Akita, Yamagata, and Fukushima. "Kanto-Koshin'etsu" : Ibaraki, Tochigi, Gunma, Saitama, Chiba, Tokyo, Kanagawa, Shizuoka, Yamanashi, Nagano, and Niigata. "Chuhbu-Hokuriku" : Toyama, Ishikawa, Fukui, Gifu, Aichi, and Mie. "Kinki" : Shiga, Kyoto, Osaka, Hyogo, Nara, and Wakayama. "Chuh-Shikoku" : Tottori, Shimane, Okayama, Hiroshima, Yamaguchi, Kagawa, Ehime, Tokushima, and Kochi. "Kyushu-Okinawa" : Fukuoka, Saga, Nagasaki, Kumamoto, Oita, Miyazaki, Kagoshima, and Okinawa.

[b] $P < 0.001$ against other areas (Chi square test).

[c] All but one were from cases of imported infection.

Table 4 HEV genotype and clinical manifestation: the severer the disease the higher the frequency of genotype 4.

HEV genotype		Disease categories: Subclinical	AH	ASH + FH
1	(n = 7)	—	6	1
2	(n = 0)	—	—	—
3	(n = 135)	52	76	7
4	(n = 78)	7	48	23
	Rate of type-4[a]	7/59(12%)	48/130(37%)	23/31(74%)

[a] $P < 0.001$ between "Subclinical" and "AH" as well as between "AH" and "ASH+FH" (Chi square test).

は1979年に発生したものであった[13]．

HEV genome 塩基配列解析

ORF1内の異なる3領域の，それぞれ69 nt[17]，326 nt[18]，821 nt[19]の全てあるいは少なくとも一つの断片をPCRで増幅し，direct sequencingすることによりgenotypeを決定した．

統計学的有意差検定

群間の比率の差や平均値の差の有意性検定の為に用いた統計学的方法は各々のTableの脚注の中に記す．

結　果

HEV感染者の居住地

居住地情報が得られた症例の地域別内訳（括弧内は例数）は，北海道（n＝130），岩手（15），宮城（1），山形（1），福島（1），茨城（4），栃木（3），群馬（1），埼玉（6），千葉（6），東京（23），神奈川（5），静岡（1），山梨（1），長野（1），新潟（2），富山（3），石川（1），愛知（3），京都（1），大阪（2），奈良（2），兵庫（5），鳥取（4），岡山（4），広島（2），愛媛（2），福岡（1），長崎（12），熊本（1），大分（2），沖縄（3），であった．

HEV感染者の性と年齢

性別情報不明あるいは病型情報不明であった11名を除いた243名に基づく，性差の成績をTable 1に示す．同様に，年齢不詳あるいは病型情報不明の12名を除外

Table 5 Liver function test levels differed by HEV genotype.

Parameters[a]	Genotype 3			Genotype 4			P
	n	mean	SD	n	mean	SD	
peak ALT (IU/L)	101	1676	1390	75	3048	2501	< 0.001[b]
peak T.B. (mg/dL)	80	7.1	8.6	71	11.8	8.9	0.01[c]
nadir P.T. (%)	74	79.6	26.3	67	63.3	27.7	< 0.001[c]

[a] Abbreviations : ALT, alanine amino transferase ; T.B., total bilirubin ; P.T., prothrombin time. [b] By Welch. [c] By *t* test.

Table 6 Month when the infection occurred, suggesting that there was no seasonality.

Month	Number of cases	Adjusted number
January	20	
February	16	
March	21	20[a]
April	37	24[b]
May	11	
June	17	
July	20	
August	22	
September	26	21[c]
October	17	
November	23	
December	18	

[a] Of the 21 cases in March, 2 were infected simultaneously by eating the same *namagimo* (raw liver) of wild boar[21] while the other 19 were exposed to respective infection-sources, and hence the number of independent infections should be 20, not 21 ; [b] Similarly, of the 37 cases in April, 11 were from a mini-outbreak that occurred after wild boar barbecue party[20] and 4 were from deer-*sashimi* sharing[22] ; [c] Of the 26 in September also included 6 individuals from a mini-outbreak[10].

した242名に基づく，年齢分布の成績をTable 2に示す．

男性優位，中高年優位が一見して顕著であるのみならず，不顕性感染群と顕性感染群(特に劇症肝炎群)との間に，年齢分布の顕著な有意差が認められた．即ち，高齢になるほど重症化率が高かった．逆に云えば，不顕性感染群には若年者が多く存在した．

Genotype 分布の地域差

HEV genotype が判明した228例について，居住地(全国を北海道，東北，関東甲信越，中部北陸，近畿，中国四国，九州沖縄の7ブロックに分割)ごとの genotype 分布を Table 3 に示す．

北海道以外の地域では genotype 3 が圧倒的多数を占めたが，北海道に於いては genotype 3 と 4 がほぼ同数存在した．Genotype 1 が検出された8名中7名はインド (n＝4)，バングラデシュ (2)，ネパール (1) への渡航歴を有していた．

HEV genotype と肝炎重症度との相関

Genotype情報及び病型診断情報の両方が得られた220例について，病型ごとの genotype 分布を Table 4 に示す．

Genotype 4 の頻度が，不顕性感染群から急性肝炎群へ，更には重症肝炎群(急性肝炎重症型＋劇症肝炎)へと有意差を以て上昇 (12%→37%→74%) していた．同様に，Table 5 に見る如く，経過中最高 ALT 値，経過中最高総ビリルビン値，経過中最延長プロトロンビン時間値のいずれもが，genotype 4 の相対的高病原性を示唆する所見を示した．

季節性

発生月が判明した247例の集計結果を Table 6 に示す．

4月が突出して高い発生例数 (n＝37) を示したが，そのうちの11例(於長崎)[20]及び2例(於鳥取)[21]は夫々同一感染源による小規模集団感染に属するものであった故，11 cases→1 incidence，2 cases→1 incidence として，互いに独立する感染発生件数をカウントし直すと，4月のそれは n＝24 に減少した．同様に，3月と9月にも夫々1件ずつの小規模集団感染事例[10][22]が含まれていた．かくて，互いに独立する感染発生件数 (Table 6 に於ける "adjusted number") で比較する限り，顕著な月別変動は存在しなかった．

Table 7　Routes of transmission.

Routes	Number of cases (%)	With direct evidence	With indirect evidence
Contact with animal	1(0.5%)	—	1[a]
Blood transfusion	5(2.3%)	5[b]	—
Travel and Import	17(7.9%)	—	13[c]
Zoonotic food-borne	68(31%)	5[d]	26[e]
Unknown	125(58%)	—	—

[a] *Patient's pet cat was anti-HEV positive* [8]. [b] *Complete matching of HEV sequences between donor and recipient* was observed in each case [11),13)]. [c] Nucleotide sequences of HEV from the patients were more homologous to those in the visited countries (India, Thailand, Nepal, Pakistan, Bangladesh, China) than those in Japan. [d] Complete matching of HEV sequences between patients and left-over animal meats [16),22)]. [e] Shown in literature [7),10),20),21)].

感染経路

感染経路を確定あるいは推定し得た症例は，全体の約40%でしかなかった（Table 7）.

5例の輸血感染（1例は愛知県，1例は東京，3例は北海道）は全て，ドナーと受血者の間でHEV RNA sequenceの一致が確認された直接証明例である．一方，他の感染経路（animal contact, travel and import, zoonotic food-borne）に於いては，感染源と感染者のHEV塩基配列が一致するとの直接証拠が得られたのは，シカからの感染[22]とイノシシからの感染[16]の2事例5名のみであって，その他は全て間接証拠からの推定である．感染源であると確定あるいは推定された動物種は，ブタ（症例数n＝44），イノシシ（15），シカ（5），動物種不明（2）であった.

北海道と本州以南で感染経路を比較すると，輸入感染の頻度に顕著な差が認められた：北海道1/130(0.8%) vs 本州以南16/124（13%）.

考　察

本邦を含む先進工業地域諸国からは初出と思われる，この200例を越えるHEV感染例の解析から得られた成績の中には，幾つかの興味深い知見が含まれている.

先ず，感染者のデモグラフィーに関しては，従来の教科書におしなべて“a disease of young adults”と記載されていたのに反し，本研究の成績は「中高年男性の病気」であることを強く示唆した．少数例ではあるが同様の成績が我国からも（A型肝炎に比較してE型肝炎患者は高齢で男性優位）[23]フランスからも（男女比約4対1，平均年齢約50歳）[24]も報告されているので，従来の教科書や常識が依拠していた流行地に於ける疫学と，これから明らかにされるであろう非流行地に於ける疫学の間に，相当の差異があるものと考えられる.

肝炎重症化の因子についても然りである．流行地に於ける観察から，妊娠第三期に於ける感染が従前唯一の重症化因子として認識されて来たが，非流行地である日本に於ける本研究の集計例の中に妊婦例は一例だに存在せず，寧ろ，加齢とHEV genotype 4が，新たな重症化因子として浮き彫りになった.

特に，HEV genotype 4とdisease severityとの間の有意な相関は，従前未報告の新知見であり，本研究の成果の中で最も特筆に値するものである．即ち，重症肝炎例にgenotype 4が多く見られるとの報告は従来から存在したが[15),24)]，病原性の強弱に関するgenotype 3と4との間の差異を統計学的有意差を以て示したのは，本報告が初めてである．両genotypesの間にはゲノム構造上も若干の差異がある（genotypes 1, 2, 3ではORF1とORF3が別フレーム上にあるが，genotype 4に於いては同一フレーム）し，増殖速度の差異（genotype 3<genotype 4）を示唆する所見（姜貞憲，松林圭二他，unpublished results）も得られているから，本研究が示唆したgenotype 4の相対的高病原性について，今後その機序が次第に明らかにされて行くと思われる.

HEV感染に於けるzoonotic transmissionの重要性は，特に我国からの多数の報告[7),8),10),14),16),20)~22),25)]により広く認識されるところとなった．その所為もあり，今回の全国集計に任意登録された症例の多くに於いては，動物由来感染を疑うための問診が相当積極的に為されていたが，それでもなお，zoonotic transmissionで説明し得る症例は全体の約30%でしかなかった．輸入感染例の約3倍もの頻度で動物由来感染が存在するということ自体が，新しく且つ刮目すべき知見ではあったものの，もっと重要な知見は，集計症例全体の約60%もが感染経路不明のまま残されたという事実の方だったかもし

れない．何故なら，それにより，我々が未だ把握していない感染経路の存在をも念頭に置いた今後の研究の必要性が示されたからである．そして，その目的の為には，特に北海道に於いて一層積極的な調査を行うことが望まれる．北海道で経験される輸入感染の頻度（0.8%）は本州以南でのそれ（13%）の10分の1以下でしかないという事実が，道内の感染源の重要性を何よりも雄弁に物語っているからである．

謝辞：本研究必要経費の大半は厚生労働省科研費肝炎等克服緊急対策研究事業からの助成金によってカバーされた．著者リストに載せ得なかった多数の研究協力者諸兄姉に深謝する．

文　献

1) 藤吉　誠，荘司貞志，近藤朝明，他．胆汁うっ滞像を認めたE型肝炎に不顕性小型アメーバ感染を合併した1例．日本消化器病学会雑誌　1992；89：2804—2807
2) 安藤文英，小柳年正，酒井好古．国内で感染し発症した散発性E型急性肝炎と思われる1邦人例．肝臓　1994；35：560—565
3) 三浦英明，古橋修介，山田春木．腸チフスによる敗血症を併発したE型急性肝炎重症型の1例．肝臓　2001；42：133—137
4) 佐藤　慎，井戸健一，磯田憲夫，他．海外渡航歴のないE型急性肝炎の1例．肝臓　2002；43：332—335
5) Mizuo H, Suzuki K, Takikawa Y, et al. Polyphyletic strains of hepatitis E virus are responsible for sporadic cases of acute hepatitis in Japan. J Clin Microbiol 2002; 40: 3209—3218
6) Takahashi M, Nishizawa T, Yoshikawa A, et al. Identification of two distinct genotypes of hepatitis E virus in a Japanese patient with acute hepatitis who had not travelled abroad. J Gen Virol 2002; 83: 1931—1940
7) Yazaki Y, Mizuo H, Takahashi M, et al. Sporadic acute or fulminant hepatitis E in Hokkaido, Japan, may be food-borne, as suggested by the presence of hepatitis E virus in pig liver as food. J Gen Virol 2003; 84: 2351—2357
8) Kuno A, Ido K, Isoda N, et al. Sporadic acute hepatitis E of a 47-year-old man whose pet cat was positive for antibody to hepatitis E virus. Hepatol Res 2003; 26: 237—242
9) 齊藤奈津子，土山寿志，大森俊明，他．北陸地方で確認された散発性E型急性肝炎の1例．肝臓　2004；45：268—273
10) 加藤　将，穐市幸二，松林圭二．焼肉店での会食後に発生したE型肝炎ウイルス集団感染：うち1例は劇症肝炎で死亡．肝臓　2004：45：688
11) Matsubayashi K, Nagaoka Y, Sakata H, et al. Transfusion-transmitted hepatitis E caused by apparently indigenous hepatitis E virus strain in Hokkaido, Japan. Transfusion 2004; 44: 934—940
12) Kumagai M, Nishii Y, Koizumi Y, et al. Domestic infection of hepatitis E in Japan. Intern Med 2004; 43: 807—810
13) Mitsui T, Tsukamoto Y, Yamazaki C, et al. Prevalence of hepatitis E virus infection among hemodialysis patients in Japan: evidence for infection with a genotype 3 HEV by blood transfusion. J Med Virol 2004; 74: 563—572
14) Hijikata S, Satoh Y, Iwashita Y, et al. A case of hepatitis E who had a history of frequent ingestion of raw meat and viscera from wild deer and boars. J Jpn Soc Gastroenterol 2005; 102: 723—728
15) Mizuo H, Yazaki Y, Sugawara K, et al. Possible risk factors for the transmission of hepatitis E virus and for the severe form of hepatitis E acquired locally in Hokkaido, Japan. J Med Virol 2005; 76: 341—349
16) Li TC, Chijiwa K, Sera N, et al. Hepatitis E virus transmission from wild boar meat. Emerging Infect Dis 2005; 11: 1258—1260
17) Takahashi K, Kang JH, Ohnishi S, et al. Full-length sequences of six hepatitis E virus isolates of genotypes III and IV from patients with sporadic acute or fulminant hepatitis in Japan. Intervirology 2003; 46: 308—318
18) Takahashi K, Kang JH, Ohnishi S, et al. Genetic heterogeneity of hepatitis E virus recovered from Japanese patients with acute sporadic hepatitis. J Infect Dis 2002; 185: 1342—1345
19) Tanaka Y, Takahashi K, Orito E, et al. Molecular tracing of Japan-indigenous hepatitis E viruses. J Gen Virol 2006; 87: 949—954
20) Tamada Y, Yano K, Yatsuhashi H, et al. Consumption of wild boar linked to cases of hepatitis E. J Hepatol 2004; 40: 869—870
21) Matsuda H, Okada K, Takahashi K, et al. Severe

hepatitis E virus infection after ingestion of uncooked liver from a wild boar. J Infect Dis 2003; 188: 944

22) Tei S, Kitajima N, Takahashi K, et al. Zoonotic transmission of hepatitis E virus from deer to human beings. Lancet 2003; 362: 371—373

23) Sainokami S, Abe K, Kumagai I, et al. Epidemiological and clinical study of sporadic acute hepatitis E caused by indigenous strains of hepatitis E virus in Japan compared with acute hepatitis A. J Gastroenterol 2004; 39: 702—703

24) Mansuy JM, Peron JM, Abravanel F, et al. Hepatitis E in the south west of France in individuals who have never visited an endemic area. J Med Virol 2004; 74: 419—424

25) 高橋和明，岡田克夫，姜　貞憙，他．重症肝炎との関連性濃厚なE型肝炎ウイルス genotype IV 内の広域分布型一系統：鳥取，新潟，札幌から得られた3本の完全長および1本の準完全長 HEV 塩基配列．肝臓　2005；46：389—390

Demographic, epidemiological, and virological characteristics of hepatitis E virus infections in Japan based on 254 human cases collected nationwide

Toshinori Abe[1], Tatsuya Aikawa[2], Yoshihiro Akahane[3], Masahiro Arai[4], Yasuhiro Asahina[5], Yoshinari Atarashi[6], Kazuaki Chayama[7], Hideharu Harada[8], Naoaki Hashimoto[9], Akiko Hori[10], Takafumi Ichida[11], Hiroki Ikeda[12], Akihisa Ishikawa[13], Takayoshi Ito[14], Jong-Hon Kang[15], Yoshiyasu Karino[16], Hideaki Kato[17], Masaru Kato[18], Mari Kawakami[19], Naoto Kitajima[20], Tsuneo Kitamura[21], Naohiko Masaki[22], Keiji Matsubayashi[23], Hiroyuki Matsuda[24], Atsushi Matsui[25], Kojiro Michitaka[26], Hiroshi Mihara[27], Katsuhiko Miyaji[28], Hiroshi Miyakawa[29], Hitoshi Mizuo[30], Satoshi Mochida[31], Mitsuhiko Moriyama[32], Shuhei Nishiguchi[33], Katsuo Okada[34], Hidetsugu Saito[35], Hiroshi Sakugawa[36], Minoru Shibata[37], Kazuyuki Suzuki[38], Kazuaki Takahashi[4], Gotaro Yamada[39], Kazuhide Yamamoto[40], Taro Yamanaka[41], Hiroaki Yamato[42], Koji Yano[43], Shunji Mishiro[4]

To know the reality of hepatitis E virus (HEV) infections in Japan, quite obscure until a few years ago, we have collected a total of 254 human cases of HEV infection, and analyzed for demographic, epidemiological, and virological characteristics. As a result, we now know [i] HEV has penetrated nationwide from Hokkaido to Okinawa; [ii] hepatitis E is a disease of middle-aged people (approx. 50 years old in average) with a predominance of male over female (approx. 3.5 vs 1); [iii] HEV strains of genotype 3 and 4 are autochthonous in Japan, but the latter is present almost exclusively in Hokkaido; [iv] the older the age the severer the disease; [v] HEV genotype 4 is associated with more obvious and severer clinical manifestations than genotype 3; [vi] no seasonality in its incidence; and [vii] transmission routes remain obscure in most cases (approx. 60%), whereas about 30%, 8%, and 2% are ascribable to zoonotic food-borne transmission, imported infection, and via blood transfusion, respectively.

Kanzo 2006; 47: 384—391

1) Isshin Hospital, 2) Aikawa Naika Hospital, 3) Kofu Municipal Hospital, 4) Toshiba General Hospital, 5) Musashino Red Cross Hospital, 6) Toyama Medical University, 7) Hiroshima University School of Medicne, 8) NHO Tokyo Hospital, 9) Tokyo Teishin Hospital, 10) Kokuho Central Hospital, 11) Juntendo University Shizuoka Hospital, 12) Kansai Medical University, 13) Hitachi General Hospital, 14) Showa University School of Medicine, 15) Teine Keijinkai Hospital, 16) Sapporo Kosei Hospital, 17) Toyokawa Municipal Hospital, 18) Hokkaido University School of Medicine, 19) Tottori University School of Medicine, 20) Kasai City Hospital, 21) Juntendo University Urayasu Hospital, 22) International Medical Center of Japan, 23) JRC Hokkaido Blood Center, 24) Matsuda Naika Clinic, 25) Shisei Hospital, 26) Ehime University School of Medicine, 27) Takaoka Municipal Hospital, 28) Osaka Medical College, 29) Teikyo University School of Medicine at Mizonokuchi, 30) Kin-ikyo Chuo Hospital, 31) Saitama Medical University, 32) Nihon University School of Medicine, 33) Hyogo Medical University, 34) Tottori Prefectural Central Hospital, 35) Keio University School of Medicine, 36) Heart-life Hospital, 37) NTT East Japan Kanto Hospital, 38) Iwate Medical University, 39) Kawasaki Medical University, 40) Okayama Saiseikai General Hospital, 41) Itabashi Chuo Hospital, 42) Kitami Red Cross Hospital, 43) NHO Nagasaki Medical Center

医薬品　研究報告 調査報告書

識別番号・報告回数			報告日	第一報入手日	新医薬品等の区分	総合機構処理欄
一般的名称	－		研究報告の公表状況	日刊薬業, 第 12105 号, 平成 18 年 9 月 6 日	公表国 日本	
販売名(企業名)	－					

研究報告の概要	使用上の注意記載状況・その他参考事項等
エイズウイルス(HIV)のうち、世界的にも感染例が少ない HIV-2 に日本人男性が初めて感染していたことがわかった。厚生労働省は、感染例の多い HIV-1 に比べ HIV-2 は感染力が弱いため、診断や治療などの体制は従来通りとする一方、HIV-2 の抗体検査の実施を徹底するよう都道府県に通知した。 HIV-2 は主に西アフリカで流行しており、感染してから発病までの期間が長いという。国内では 1993 年に韓国籍の 2 人の感染が確認されたが、今まで日本人の感染例は無かった。 男性は気管支喘息を患い、国内の医療機関に入院し、検査の結果、HIV-2 に感染していることが判明した。過去に西アフリカに渡航し、現地で輸血した経験があるため、これが感染経路と見られている。男性は既に症状が改善し退院している。 入院先の医療機関から依頼を受けた厚生労働省研究班が検査を行い、遺伝子検査の結果、HIV-2 であると確定、8 月 11 日に厚生労働省に報告した。 HIV 抗体検査で陽性だった場合には、抗体の種類を判別する確認検査を行うが、国内感染者のほとんどが HIV-1 のため、HIV-1 の検査だけをして HIV-2 の検査を行わない医療機関もあり得るため、厚生労働省では 8 月 11 日付で各都道府県に対し、HIV-2 の検査も確実に行い、検査漏れがないよう通知した。	「重要な基本的注意」に原材料となる血液について抗 HIV-2 抗体陰性を確認している旨を記載。

報告企業の意見	今後の対応
世界的にも感染例の少ない HIV-2 に感染した初の日本人男性に関する報告である。当社血漿分画製剤は原料血漿の段階で抗 HIV-2 抗体陰性を確認している。また、HIV に対するウイルスクリアランス指数が 9 以上であることを確認しているので、安全性について特に問題ないと考えられる。	今後とも HIV-2 に関する安全性情報等に留意していく。

厚労省　日本人初のＨＩＶ２型感染で検査徹底を通知

エイズウイルス（ＨＩＶ）のうち、世界的にも感染例が少ないＨＩＶ２型に日本人男性が初めて感染していたことが４日分かった。厚生労働省は、感染例の多いＨＩＶ１型に比べ２型は感染力が弱いため、診断や治療などの体制は従来通りとする一方、２型の抗体検査の実施を徹底するよう都道府県などに求めている。

２型は主に西アフリカで流行しており、１型に比べ感染力が弱く感染してから発病までの期間が長いという。国内では１９９３年12月と２００２年１月に韓国籍の２人の感染が確認されたが、今まで日本人の感染例はなかった。

男性は気管支喘息を患い、国内の医療機関に入院し、検査の結果、ＨＩＶ２型に感染していることが判明した。過去に西アフリカに渡航し、現地で輸血をした経験があるため、これが感染経路とみられている。男性はすでに症状が改善し退院している。

入院先の医療機関から依頼を受けた厚労省研究班が検査を行い、遺伝子検査の結果、２型であると確定、先月11日に厚労省に報告した。ＨＩＶ抗体検査で陽性だった場合には、抗体の種類を判別する確認検査を行うが、国内感染者のほとんどが１型のため、１型の検査だけをして２型の検査を行わない医療機関もあり得る。このため厚労省では先月11日付で各都道府県に対し、２型の検査も確実に行い、検査漏れがないよう通知した。

先端医療振興財団　助成事業の概要を公表

神戸市の先端医療振興財団は５日、２００７年度から実施する、「がん情報普及・啓発に関する講演会等への助成事業」の公募に関する概要を公表した。財団傘下の神戸臨床研究情報センター（神戸ＴＲＩ）の活動の一環で、１件当たり50万円、年間総額２５０万円を計上した。助成対象は、07年度に行われる市民・患者を対象にした、がん情報の普及啓発に関する講演会やシンポジウム。応募対象団体は日本がん患者団体協議会（ＪＣＰＣ）に加盟し、推薦を受けたＮＰＯ（民間非営利団体）などの民間団体。同一団体からの複数応募はできない。助成先は財団の審査委員会で決める。公募申し込み受付期間は９月15日～11月15日。詳細は、財団ホームページ（http://www.ibri-kobe.org/）で。

テムリック　第１種医薬品製造販売業許可を取得

ＣＲＯ（医薬品開発業務受託機関）のテムリックは４日、東京都から「第１種医薬品製造販売業」の業許可を８月24日付で取得したと発表した。ＣＲＯ業務とは別に行っていた、自社開発品の承認申請のために取得した。テムリックはがん領域に特化したＣＲＯとして２００２年に事業を開始したが、04年に「ＴＭ－４１１」（多発性骨髄腫）の開発権・販売権を導入し、創薬事業を始めた。同剤は現在治験実施中で、終了は来年以降になる見通し。今年４月にはすでに２品目としてシエーリングからがん治療薬の導入契約を締結し、７月末に「ＴＭ－５１１」として治験届を提出した。

医薬品 研究報告 調査報告書

識別番号・報告回数			報告日	第一報入手日	新医薬品等の区分	機構処理欄
				2006. 8. 22	該当なし	

一般的名称	洗浄人赤血球浮遊液	研究報告の公表状況		公表国
販売名(企業名)	洗浄赤血球「日赤」(日本赤十字社) 照射洗浄赤血球「日赤」(日本赤十字社)		H Cordel, I Quatresous, C Paquet, E Couturier. Eurosurveillance weekly release: 2006,volume 11, 8, 2006 Aug 10.	インド

研究報告の概要

○インドにおけるチクングンヤの再興:高まる脅威
チクングンヤウイルス(CHIKV)の感染がインドで拡大している。2005年12月以降最も被害の大きい5つの州から896,500人以上のチクングンヤ疑い症例が報告されている。北部の州からは1例も報告されていない。2004年末以降、チクングンヤはインド洋の南西部の島々で流行し、その後マダガスカルとインドでも報告された。インドにおけるチクングンヤは、1963年にコルカタで最初に検出された。1973年のインド西部での流行以降はサーベイランスは実施されておらず、インドから消滅したと考えられてきた。最近の研究では発症患者の約50%がRT-PCRでRNA陽性だったが、実際の発生率ははるかに高いと考えられる。病院に行かない患者が多く、受診してもRNA陽性となるのは1日目から4日目のみで臨床検査は難しい。症状は、38.5~40℃の高熱、筋肉痛、頭痛、関節の腫れと激痛、発症5日以降のかゆみを伴う斑点状丘疹で、多くは自己限定的で1~10日持続した。関節痛は症例の約10%で3週間以上持続し、数ヶ月~数年間続くこともある。温暖湿潤な気候と貯水池は媒介蚊の繁殖に適した環境で、貧しい人々はより感染しやすくなっている。インド洋のCHIKV分離株の遺伝子構造はウイルスが急速に変異することを示唆している。疾患は自己限定的であるが、流行地域への渡航者の感染リスクは引き続き存在する。輸入症例が欧州の多くの国から報告されており、フランスでは2006年3月に血液暴露によると考えられる国内感染例が発生している。媒介蚊の一つであるヒトスジシマカは欧州でも見られるため、ウイルス流入と地域内/持続感染のリスクについてさらに調査が必要である。
抗ウイルス剤とワクチンの開発が急務である。さらなる感染拡大を抑えるための対策の強化が求められる。臨床管理を改善する方法、特に早期検出、患者の栄養補給、その他の予防手段によって症状を大きく緩和できる。

報告企業の意見	今後の対応
チクングンヤウイルスの感染がインドで拡大しており、ヨーロッパへのウイルス流入と地域内感染が危惧されるとの報告である。	日本赤十字社では、輸血感染症対策として問診時に海外渡航歴の有無を確認し、帰国後4週間は献血不適としている。今後も引き続き、新たなウイルス等による感染症の発生状況等に関する情報の収集に努める。

使用上の注意記載状況・その他参考事項等

洗浄赤血球「日赤」
照射洗浄赤血球「日赤」

血液を介するウイルス、細菌、原虫等の感染
vCJD等の伝播のリスク

Resurgence of chikungunya virus in India: an emerging threat

SK Saxena[1] (shailen@ccmb.res.in), M Singh[1], N Mishra[1], V Lakshmi[2]

[1]Centre for Cellular and Molecular Biology, Hyderabad, India
[2]Department of Microbiology, Nizam's Institute of Medical Sciences, Hyderabad, India

Since December 2005, an outbreak of chikungunya virus (CHIKV) infection has been ongoing in various states of India (Karnataka, Maharashtra, Andhra Pradesh, Tamil Nadu, Madhya Pradesh, Gujarat, Orissa and Kerala) with potential spread to neighbouring states [1,2]. Cases were first recognised and reported in December 2005. In July 2006, India's National Vector Borne Disease Control Programme (NVBDCP) reported a reduction in the number of cases in the affected districts while other districts are now becoming affected for the first time. The spread is of unprecedented magnitude and over 896 500 suspected chikungunya cases have been reported since December 2005 from the five worst affected states (Andhra Pradesh, Karnataka, Maharashtra, Tamil Nadu and Madhya Pradesh) [3]. No chikungunya cases have been reported from the northern states.

Recent large-scale outbreaks of fever caused by CHIKV infection in India have confirmed the reemergence of chikungunya in this part of Indian subcontinent. Since the end of 2004, chikungunya has emerged in the islands of the southwestern Indian Ocean (Comoros, Mauritius, Seychelles and Reunion), where several hundred thousand cases have been reported. Chikungunya was later also reported in Madagascar and in India [4,5]. Chikungunya is not new to the Indian subcontinent. Since it was first detected in Calcutta in 1963 [6], there have been reports of CHIKV infection in different parts of India [7,8,9]. Previously, the most recent Indian chikungunya outbreak was reported in 1973 in western India, in Barsi, Sholapur district, Maharashtra state [10]. Subsequently, there has been no active or passive surveillance carried out in India and it was believed that chikungunya had disappeared from the Indian subcontinent [11,12].

A recent study looked at samples taken from over 140 symptomatic patients with clinical picture of chikungunya who were presented to the Nizam's Institute of Medical Sciences hospital in Hyderabad (the capital of Andhra Pradesh) in March and April 2006. About 50% were found positive for the presence of CHIKV specific RNA (through demonstration of the virus-specific 500 bp amplicon) by reverse transcription-polymerase chain reaction (RT-PCR) [V Lakshmi et al, unpublished data]. However, the true incidence is thought to be much higher, because due to the self-limiting nature of the illness a large proportion of patients did not go to hospital, and even for those who did, laboratory diagnosis proved difficult as RT-PCR was positive for the virus in samples collected between the first and fourth day only, indicating the viraemic phase of the infection. Most patients with acute CHIKV infection presented with high fever (ranging from 38.5°- 40°C), muscle pain, headache and swelling and severe pain in the joints with polyarthralgia (pain in several joints) followed by an itching maculopapular rash five days after onset. Symptoms were generally self-limiting and lasted 1-10 days. Almost 10% of cases reported had prolonged joint pain for more than three weeks. However, joint pain may persist for several months or years. Females were more affected than males, a feature probably associated with the daytime and indoor feeding habits of the mosquito vector in India, *Aedes aegyptii*. All age groups were evenly represented.

Warm, humid climates and water reservoirs serve as an excellent breeding ground for the vector of the virus, *Aedes* mosquitoes. With an increase in temperature, susceptibility of mosquitoes to CHIKV increases [13]. High population density, lack of adequate resources for vector control and hygiene added to the vulnerability of poor people to chikungunya infection. The unique molecular features of the recently analysed Indian Ocean isolates of CHIKV [4] suggest that the virus can evolve rapidly. Studies are in progress to confirm genomic structure and virulence of the recent CHIKV from India.

Although the disease is self-limiting, the risk to non-immune travellers from other parts of the world to areas with a chikungunya epidemic, including India, continues to exist and should be included in the differential diagnosis of travellers returning home with fever. The magnitude of this risk cannot be precisely determined at this time. There is a risk of importing the virus to Europe from affected parts of the world, including Africa and South East Asia, where the virus is endemic. Imported cases have been reported from a number of European countries, including an autochthonous case from France in March 2006, probably contaminated through a blood exposure incident [14]. Considering the extent of the current chikungunya outbreak, the risk of introduction and autochthonous/sustained transmission of the virus in Europe needs further investigation, because one vector, the tiger mosquito *A. albopictus*, is also present in Europe and could increase the likelihood of its future autochthonous transmission in these countries. Various recommendations have been suggested by European experts to ensure the measures to prevent the emergence of imported viral diseases are strengthened in

Europe [5,15]. Pregnant women, families with young children, older people, and those with significant comorbidity should be advised to consult their physician before travelling to the Indian subcontinent, and travellers should be informed about the magnitude of the risk of contracting the disease and decide according to their own judgment. There are no specific preventive medications or vaccines for chikungunya fever, but there are steps travellers can take to reduce risk of being bitten by infected mosquitoes [15]. Despite infecting millions of people worldwide, chikungunya infection has been neglected since its discovery. Worldwide, there are a number of other infections with mosquito-transmitted viruses (arboviruses) with similar symptoms which may be confused with chikungunya, such as Sindbis, Ross River and dengue, and these, together with a detailed travel history, should be considered in the differential diagnosis in returning travellers.

Considering high number of cases, and lack of specific antiviral therapy, it is imperative that specific antiviral agents and vaccine be developed. Although the disease is self-limiting, sustained and intensified control measures (such as regular fogging with pesticides, awareness of the disease and vector, detection and elimination of vector breeding sources, proper facilities for health care and community awareness about the prophylactic measures) are required to control the further spread of the disease. The government of India has taken up necessary steps, in accordance with the NVBDCP guidelines on reducing mosquito breeding sources, use of temephos larvicide in recommended doses, the release of larva-eating fish (*Gambusia*) into the wells and the water bodies to control the mosquito menace and deployment of mobile teams (three teams per district in the affected districts, consisting of epidemiologists, public health specialists, microbiologists and entomologists for assessment of the situation and providing technical assistance and guidelines) and mobilisation of health workers and volunteers [16,17]. Finally, measures to improve clinical management, especially early detection, nutritional support to the affected patients, and other preventive measures may largely mitigate the disease. The wider issues of ecology, current agricultural practices, water management systems, and human behaviour patterns will need to be reviewed. This requires a combination of strategies and we need to proceed with a sense of urgency in this matter.

References:

1. WHO Epidemic and Pandemic Alert and Response (EPR). Chikungunya and Dengue in the south west Indian Ocean. Disease Outbreak News, 17 March 2006. (http://www.who.int/csr/don/2006_03_17/en)
2. Four states affected by Chikungunya, says government. NewKerala.com website. 26 July 2006. (http://www.newkerala.com/news3.sphp?action=fullnews&id=28694) [accessed 01 August 2006]
3. Chikungunya - India (04). in: ProMED-mail [online]. Boston US: International Society for Infectious Diseases, archive no. 20060801.2120, 1 August 2006. (http://www.promedmail.org)
4. Schuffenecker I, Iteman I, Michault A, Murri S, Frangeul L, Vaney MC, et al. Genome microevolution of chikungunya viruses causing the Indian ocean outbreak. PLoS Med 2006; 3(7): e263. Epub 2006 May 23. (http://www.pubmedcentral.gov/articlerender.fcgi?tool=pubmed&pubmedid=16700631)
5. Depoortere E, Coulombier D. ECDC Chikungunya risk assessment group. Chikungunya risk assessment for Europe: recommendations for action. Euro Surveill 2006; 11(5): E060511.2. (http://www.eurosurveillance.org/ew/2006/060511.asp#2)
6. Shah KV, Gibbs CJ Jr, Banerjee G. Virological investigation of the epidemic of haemorrhagic fever in Calcutta: isolation of three strains of Chikungunya virus. Indian J Med Res 1964; 52: 676-83.
7. Jadhav M, Namboodripad M, Carman RH, Carey DE, Myers RM. Chikungunya disease in infants and children in Vellore: a report of clinical and haematological features of virologically proved cases. Indian J Med Res 1965; 53: 764-76.
8. Dandawate CN, Thiruvengadam KV, Kalyanasundaram V, Rajagopal J, Rao TR. Serological survey in Madras city with special reference to chikungunya. Indian J Med Res 1965; 53: 707-14.
9. Thiruvengadam KV, Kalyanasundaram V, Rajgopal J. Clinical and pathological studies on chikungunya fever in Madras city. Indian J Med Res 1965; 53: 729-44.
10. Padbidri VS, Gnaneswar TT. Epidemiological investigations of chikungunya epidemic at Barsi, Maharashtra state, India. J Hyg Epidemiol Microbiol Immunol 1979; 23(4): 445-51.
11. Pavri K. Disappearance of Chikungunya virus from India and South East Asia. Trans R Soc Trop Med Hyg 1986; 80: 491.
12. Ravi V. Re-emergence of Chikungunya virus in India. Indian J Med Microbiol 2006; 24: 83-4. (http://www.ijmm.org/article.asp?issn=02550857;year=2006;volume=24; issue=2;spage=83;epage=84;aulast=Ravi)
13. Mourya DT, Yadav P, Mishra AC. Effect of temperature stress on immature stages and susceptibility of Aedes aegypti mosquitoes to chikungunya virus. Am J Trop Med Hyg. 2004; 70(4): 346-50.
14. Cordel H, Quatresous I, Paquet C, Couturier E. Imported cases of chikungunya in metropolitan France, April 2005 - February 2006. Euro Surveill. 2006; 11(4): E060420.3. (http://www.eurosurveillance.org/ew/2006/060420.asp#3)
15. Chikungunya fever - information for travellers [Fact sheet]. European Centre for Disease Prevention and Control. 17 July 2006. (http://www.ecdc.eu.int/outbreaks/Chikungunya_reunion.php)
16. AP data shows Chikungunya declining. Sify Cities website. 27 June 2006. (http://sify.com/cities/hyderabad/fullstory.php?id=14236355) [accessed 22 July 2006]
17. Efforts on to control chikungunya. The Hindu. 14 July 2006. (www.hindu.com/2006/07/14/stories/2006071405140300.htm) [accessed 8 August 2006]

back to top

医薬品

医薬品　研究報告　調査報告書

化粧品

別紙 3-13

識別番号・報告回数		回	報告日 年　月　日	第一報入手日 2006 年 8 月 8 日	新医薬品等の区分 該当なし	総合機構処理欄
一般的名称			研究報告の公表状況	Simian foamy virus infection by whole-blood transfer in rhesus macaques: potential for transfusion transmission in humans Khan, A. S. and Kumar, D. Transfusion, 46, 1352-1359 (2006).	公表国 米国	
販売名（企業名）						

研究報告の概要	使用上の注意記載状況・その他参考事項等
アカゲザルでの輸血によるサル免疫不全ウイルス (SFV) の伝播が実験的に示されている。2 頭の自然感染したサルをドナー (D1 及び D2 と命名) として用い，各ドナーあたり 2 頭のレトロウイルス陰性サルに全血を輸血したところ，D1 から輸血された 2 頭のサルだけが感染した (追跡期間は輸血後 1 年間)。感染は以下の方法で証明された。 i) 輸血されたサルにおける特定 SFV 抗体の発現 ii) 感染ドナーサルの末梢血単核細胞 (PBMNCs) からの SFV 特有配列の PCR 増幅 iii) 末梢血単核細胞 (PBMNCs) からの感染及び複製 SFV の分離 興味深いことに，ドナーのサルはそれぞれ異なる複製動態を持つ SFV 菌株に感染していた (D1 の SFV は D2 の SFV よりも複製速度が早く，D2 は D1 より中和抗体価が顕著に高かった)。また，その他の要因，例えばウイルス接種量なども感染症の伝播において重要であると示唆された。	BYL-2006-0240 Detection and molecular characteri-zation of foamy viruses in Central African chimpanzees of the Pan troglo- dytes troglodytes and Pan troglodytes vellerosus subspecies. Calattini, S. et al, J. Med. Primatol. 35, 59-66 (2006).

報告企業の意見	今後の対応
これまで，非ヒト霊長類では SFV 感染による病変の報告は無かった。また，ヒトの感染例もほとんどなかった。しかし，SFVs は，in vitro で高い細胞変性を示し，ヌクレオチド配列は宿主ゲノム内で安定して取り込まれる。現時点では，ヒト間で SFV の伝播が起こるかは明らかではない。	現時点で新たな安全対策上の措置を講じる必要は無いと考える。引き続き関連情報の収集に努める。

Simian foamy virus infection by whole-blood transfer in rhesus macaques: potential for transfusion transmission in humans

Arifa S. Khan and Dhanya Kumar

BACKGROUND: Cross-species infection of humans with simian foamy virus (SFV) has been reported in European and North American nonhuman primate (NHP) handlers, primarily due to wound injuries involving infected animals in research centers and zoos. Additionally, African hunters have been found to be infected with SFV by exposure to body fluids, blood, or tissues of infected NHPs in the wild. The persistence of infectious virus in peripheral blood mononuclear cells (PBMNC) and the recent identification of some infected blood donors has raised safety concerns regarding potential virus transmission by blood transfusion.

STUDY DESIGN AND METHODS: SFV infection by blood transfusion was evaluated by whole-blood transfer from two naturally-infected rhesus macaques (designated as D1 and D2) to retrovirus-free monkeys. Blood from D1 was transfused to two recipient monkeys R1 and R2 and from D2 to monkeys R3 and R4. Virus transmission was evaluated by immunoassays, polymerase chain reaction assays, and coculture of PBMNC for SFV isolation.

RESULTS: SFV infection was seen in R1 and R2 based on development of virus-specific antibodies, identification of SFV sequences in monkey PBMNC, and isolation of infectious virus from PBMNC. Furthermore, both R1 and R2 remained SFV-positive at about 1 year after transfusion, which was the last time tested. No evidence of SFV infection was seen in R3 and R4.

CONCLUSION: SFV transmission in macaques occurred by transfusion of blood from one of two infected donor animals. These results indicate the potential of SFV transfusion transmission in humans, which may depend on virus-specific or donor-related factors.

Cross-species transmission of retroviruses to humans is an important public health concern as exemplified by the origin of human immunodeficiency virus (HIV) from simian immunodeficiency virus (SIV).[1] The extensive use of nonhuman primates (NHPs) in biomedical research and broad exposure to infected animals in the wild has facilitated cross-infection of humans with simian foamy virus (SFV), which is highly prevalent in all NHP species and possesses a broad host range and cell tropism.[2-4] The first human transmission was reported in 1971 due to injury by an infected chimpanzee.[5] Reports of cross-species human infection with SFV have increased since the mid-1990s[6-9] and the use of more sensitive detection assays have further indicated additional NHP handlers infected with SFV due to injury incurred by infected animals[10-12] as well as identification of people infected in Africa due to exposure to body fluids and meat while hunting and butchering of NHPs.[13]

It is noteworthy that although infectious virus has been demonstrated to persist long-term in human cells, in vivo and in vitro[6,14,15] there is, thus far, no report of disease associated with SFV and no evidence of SFV transmission between humans.[6]

The persistence of stably integrated, infectious retrovirus sequences in human peripheral blood cells raises

ABBREVIATIONS: CPE = cytopathic effect; IUPM = infectious units per million total PBMNC; NHP(s) = nonhuman primate(s); PBST = phosphate-buffered saline with 0.05 percent Tween; PBST+5 percent = PBST plus 5 percent milk; SFV = simian foamy virus; SIV = simian immunodeficiency virus; RT = reverse transcriptase.

From the Laboratory of Retrovirus Research, Division of Viral Products, Center for Biologics Evaluation and Research, U.S. Food and Drug Administration, Bethesda, Maryland.

Address reprint requests to: Arifa S. Khan, PhD, 8800 Rockville Pike, HFM-454, Building 29B, Room 4NN10, Bethesda, MD 20892; e-mail: arifa.khan@fda.hhs.gov.

Received for publication March 14, 2005; revision received December 23, 2005, and accepted December 24, 2005.

doi: 10.1111/j.1537-2995.2006.00862.x

TRANSFUSION 2006;46:1352-1359.

concerns, however, regarding the safety of blood transfusion from SFV-infected blood donors. In fact, testing of archived sera identified six SFV-seropositive blood donors.[10] A retrospective study of four recipients of blood components (red cells [RBCs], filtered RBCs, and platelets [PLTs]) from one infected blood donor failed to demonstrate SFV infection; however, it was noted that additional studies are warranted to further evaluate the potential risk of SFV transmission by blood transfusion.[16] This is especially important since transmission by transfusion has been demonstrated as an important mode of acquisition of infections in humans with other retroviruses.[17,18] In this article, we have examined SFV transmission by whole-blood transfusion in a monkey model. Blood from SFV-infected donor animals was transfused into retrovirus-free monkeys, which were analyzed for SFV infection and persistence. This study evaluates the potential human risk of SFV infection by infected blood donors.

MATERIALS AND METHODS

Monkeys and blood transfusion

SFV-negative blood recipients were juvenile, rhesus macaques (*Macaca mulatta*) that were obtained from a group of animals in a domestic breeding colony (LABS of Virginia, Morgan Island, SC), which were free of SIV, simian T-lymphotropic virus, and simian retrovirus. Animals were identified as SFV-negative with a dot blot antibody assay[19] (Simian Diagnostic Laboratory, San Antonio, TX) and shipped in individual cages to the FDA animal facility (National Institutes of Health, Bethesda, MD). All animals were maintained in accordance with the *Guide for the Care and Use of Laboratory Animals* [20] under an approved protocol by the Institute Animal Care and Use Committee. The animals were housed in single cages and in a separate room from the SFV-infected blood donor monkeys. Only animals that were confirmed SFV-negative by serology and by polymerase chain reaction (PCR) analysis of peripheral blood mononuclear cell (PBMNC) DNA at the time of study initiation were used in the study. A control animal was housed in the same room as the blood recipient animals to demonstrate absence of cross-contamination due to housing and handling of the animals.

Donor animals, RhK3T and RhA2V (designated as D1 and D2, respectively, in this article) were adult rhesus macaques, naturally-infected with SFV that were maintained in single housing and in a separate room from SFV-negative animals. Donor animals were well characterized: SFV from D1 and D2 (designated as SFV-D1 and SFV-D2, respectively) were previously isolated from monkey PBMNCs and characterized in replication studies to evaluate virus fitness and nucleotide sequences were determined in the long terminal repeat region. The status of SFV infection in D1 and D2 was confirmed by serology and virus isolation from samples stored on day of blood transfer.

Blood was collected under sedation with ketamine hydrochloride (10 mg/kg). Before transfusion, blood was collected in anticoagulant (heparin or ethylenediaminetetraacetate [EDTA]) from the donor and recipient animals for preparation of PBMNC, plasma, and serum. At the time of transfusion, blood was collected in EDTA for additional PBMNC and plasma preparation and in separate tubes for blood chemistry and hematology. For blood transfer, blood (20 mL) was collected in heparin (1000 U, 1 mL, Elkins-Sinn Inc., Cherry Hill, NJ) from D1 for transfusion (10 mL each) with a butterfly catheter into the right saphenous vein of two recipient monkeys, RhCK2T and RhCK3H (designated as R1 and R2, respectively, in this article). Each animal was separately handled, and mats were changed in between each animal. Similarly, blood from D2 was transferred to RhCJ3K and RhCJ52 (designated as R3 and R4, respectively, in this article). After the blood transfer, 10 mL of saline was injected into a "housing control" animal RhOVG. Monkeys were monitored for healthy recovery after the blood transfusion based on temperature, heartbeat, and respiratory rate. After transfusion, blood was collected at various times in EDTA for PBMNC and plasma preparation for analysis of virus infection. Additionally, at each time of blood collection, serum chemistry and hematology were performed (Antech, Lake Success, NY).

Detection assays for SFV antibodies

SFV-specific antibody was detected by dot blot immunoassay[19] performed by the Simian Diagnostic Laboratory. The samples from each animal were collected and stored for concurrent analysis in the same assay.

SFV-seropositive animals were confirmed by Western blot analysis. Cell lysates were prepared from uninfected and SFV-2-infected *Mus dunni* cells (wild mouse fibroblasts; ATCC, Manassas, VA) as previously described.[21] Protein concentration was determined with a protein assay dye (Bio-Rad, Hercules, CA). Sixty micrograms of protein was heat-denatured and analyzed on an 8 percent Tris-glycine gel (Novex, San Diego, CA), run 1.5 hours at 125 V (Novex X-cell II system, Novex, San Diego, CA) in 1× Tris-glycine running buffer (24.8 mmol/L Tris, 192 mmol/L glycine, 0.1 percent sodium dodecyl sulfate). Proteins on the gel were transferred to nitrocellulose membrane (Invitrogen, Carlsbad, CA; 0.45 μm) at 30 V for 1 hour in 24.8 mmol per L Tris, 192 mmol per L glycine, 20 percent methanol. The membrane was cut into strips so that each strip contained 5 μg of protein. The strips were placed, protein side up, in individual wells of a plastic tray; rinsed at room temperature for 5 minutes each with Ultrapure water, phosphate-buffered saline (PBS) without Ca^{2+}-Mg^{2+}, PBS (pH 7.3)-0.05 percent Tween (designated as PBST); and blocked overnight at room temperature in PBST containing 5 percent nonfat dried milk (designated as

PBST+5%). The strips were then incubated with 1:100 dilution in PBST+5 percent of plasma or serum, except in case of monkey D1 where 1:500 dilution of plasma was used. The membrane strips were initially incubated for 2 hours at room temperature and then overnight at 4°C on a rocker. The strips were brought to room temperature and washed three times for 5 minutes each in PBST+5 percent and then incubated for 2 hours at room temperature with a 1:500 dilution in PBST+5 percent of horseradish peroxidase (HRP)-conjugated goat anti-monkey IgG (Cappel-ICN Pharmaceuticals Inc., Aurora, OH). The strips were then washed five times for 5 minutes each in PBST, and the protein bands were visualized by chemiluminescence with a substrate system (Supersignal CL-HRP substrate system, Pierce, Rockford, IL). The substrate was added to the membrane strips for 2 minutes, the strips then blotted with paper (Whatman 3 MM, Maidstone, Kent, England) to remove excess substrate and exposed for various times ranging from 5 seconds to 2 minutes with film (BioMax MR, Kodak, Rochester, NY).

Neutralizing antibody endpoint titers were determined in assays with homologous virus (SFV-D1 with plasma from D1 and SFV-D2 with plasma from D2). MRC-5 cells (ATCC CCL-171; human lung fibroblast) were planted in a 24-well plate with 30,000 cells per well (Passage 25) in Eagle's minimum essential medium (modified) with Earle's salt without L-glutamine (Cellgro, Mediatech, Herndon, VA) containing 10 percent heat-inactivated fetal bovine serum (FBS; Hyclone, Logan, UT), 2 mmol per L glutamine, 250 U of penicillin per mL, 250 µg of streptomycin per mL, 1× nonessential amino acids (MEM-NEAA 100×, Quality Biological, Inc., Gaithersburg, MD) 1 mmol/L sodium pyruvate in a total volume of 2 mL. Cells were incubated overnight at 37°C, and 0.2 mL was removed to replace with test sample. Monkey plasma (heat-inactivated) was diluted twofold (ranging initially from 1:50 to 1:1600) in PBS, pH 7.4, without calcium and magnesium (Quality Biological, Inc.), initially ranging from 1:50 to 1:1600. Plasma samples were incubated for 1 hour at room temperature with equal volume of SFV (100 $TCID_{50}$ per 0.1 mL), after which 0.2 mL was removed and added to each well, in triplicate. The tray was incubated at 37°C and cells observed for cytopathic effect (CPE) up to Day 13, when the final results were recorded. The antibody endpoint was the highest dilution of plasma that inhibited CPE in all replicate wells.

DNA preparation and PCR analysis

Cryopreserved PBMNCs were recovered in RPMI and washed with cold PBS (without Ca^{2+} and Mg^{2+}), and DNA was prepared with a DNA blood mini kit according to the manufacturer's protocol (QIAamp, Qiagen, Valencia CA) except that all spins were at 15,800 × g at room temperature and the DNA elution time was increased to 5 minutes at room temperature. DNA was aliquoted and stored at −80°C.

SFV sequences in PBMNC DNA were amplified by PCR with previously described conditions with set B outer primer pair and inner primer pair (3+5 and 6+7, respectively[21]). The sensitivity of the outer primer set was shown to be 10 viral copies in 10^5 cell equivalents of cellular DNA. The identity of the SFV sequences was confirmed by nucleotide sequence analysis of gel-purified DNA fragments (gel DNA recovery kit, Zymoclean, Orange, CA), obtained with primers 6 and 7. PCR primers, which amplified an 838-bp fragment of the human β-actin gene (Clontech, Palo Alto, CA), were used as a control for the presence of DNA in the sample. The PCR mixture without DNA was used as the negative control.

Nucleotide sequence analysis

Nucleotide sequence reactions were set up with primers 6 and 7, according to the protocol with a cycle sequencing kit according the manufacturer's protocol with 5X sequencing buffer (BigDye Terminator Version 3.1 cycle sequencing kit, Applied Biosystems, Foster City, CA). The sequence reactions were purified with spin columns (CentriSep, Princeton Separations, Adelphia, NJ), and sequences were determined with a DNA sequencing system (ABI Prism 377, Perkin-Elmer Applied Biosystems, Foster City, CA).

Blood processing and virus isolation

PBMNC and plasma were prepared from blood containing EDTA as preservative (SeraCare Bioservices, previously BBI Biotech Research Laboratories, Inc., Gaithersburg, MD). Plasma was aliquoted and stored at −80°C. PBMNC were prepared by the Ficoll-Hypaque method, aliquoted, and cryopreserved. For virus isolation, PBMNC were stimulated in a 24-well plate with 5 µg per mL phytohemagglutin (PHA; Murex Biotech Ltd, Dartford, Kent, England) for 72 hours in RPMI containing 10 percent (1000 U) human interleukin-2 (Roche, Indianapolis, IN), 10 percent FBS (heat-inactivated 56°C for 30 min; Hyclone), 2 mmol per L glutamine, 250 U of penicillin per mL, 250 µg of streptomycin per mL. PHA-stimulated PBMNC were added to *M. dunni* cells (1.3×10^6-1.9×10^6) in a 75-cm^2 flask for coculture in Dulbecco's minimum essential medium containing 10 percent FBS, 2 mmol per L glutamine, 250 U of penicillin per mL, 250 µg of streptomycin per mL in a total volume of 20 mL. Cultures were passaged every 3 or 4 days when the cells reached confluency and maintained until culture termination due to extensive CPE or at least 30 days. PBMNC were added back to the cultures for three passages after the initial coculture. Filtered supernatants were collected and stored at various times during the culture period for Mn^{2+}-dependent reverse transcriptase (RT)

assay.[21] SFV identity was confirmed at culture termination by PCR amplification and nucleotide sequence analysis.

PBMNC viral load determination

MRC-5 cells were planted overnight as described above for the neutralization assay. One-milliliter of medium was removed and replaced with 1 mL containing fivefold serially diluted monkey PBMNC ranging from 1×10^6 cells per mL to 320 cells per mL per well. Each dilution was tested in at least four replicates. The plate was incubated at 37°C for 14 days. Filtered supernatant was collected and analyzed for SFV by a PCR-enhanced RT assay (STF-PERT[22]). The $TCID_{50}$ was calculated by the Kärber method[23] and infectious units per million total PBMNC (IUPM) expressed as the reciprocal of the $TCID_{50}$.

RESULTS

SFV infection occurred in two recipient monkeys (R1 and R2) that were transfused with blood from donor animal D1, but not in the two animals (R3 and R4) that received blood from donor animal D2 or in a saline-injected control animal.

Detection of SFV-specific antibodies in transfused monkeys

Plasma from study animals was analyzed for SFV-specific antibodies at various times after transfusion. The results of dot blot assays are shown in Table 1. The earliest time at which SFV antibody was detected in R1 and R2 was 22 and 16 weeks, respectively, after which time both animals remained positive. The control animal was negative at all tested times.

The antibody status of the animals was further evaluated by Western blot analysis. The results in Fig. 1 indicate the presence of SFV antibodies as early as Week 1, which decreased over time, representing passive transfer of donor antibodies. The resurgence of antibodies was seen at Week 22 in R1 and at Week 16 in R2 indicating the development of antibodies in response to virus infection after transfusion. Antibodies to SFV proteins persisted at Week 48, the last time point tested: the 65K and 70K proteins most likely correspond to the diagnostic Gag doublet seen in all infected species (p68/71[24]). Passive antibody transfer also occurred in R3 and R4 after blood transfusion from D2; however, there was no evidence of new antibody development due to virus infection (data not shown). No SFV-specific antibodies were seen in the control animal.

Detection of SFV sequences in monkey PBMNC

The kinetics of SFV infection by blood transfer were evaluated by PCR analysis of monkey PBMNC DNA. SFV-specific primers amplified a 349-bp fragment from R1 and R2 from PBMNC at Week 8 after transfusion and thereafter (Fig. 2). The expected size β-actin fragment was seen in all the samples, indicating the presence of intact DNA in the samples. The identity of the PCR-amplified fragment from

TABLE 1. Development of SFV-specific antibodies by blood transfusion*

Weeks after transfusion	Monkeys		
	R1	R2	Control
0	–	–	–
1	–	–	–
2	–	–	–
4	–	–	–
8	–	–	–
11	–	–	–
16	–	+/–	–
22	+	+	–
30	+	+	–

* All samples were run in the same assay, and each sample was analyzed in two independent assays. Differences in the results in the two assays are indicated. Negative is less than 1:5.

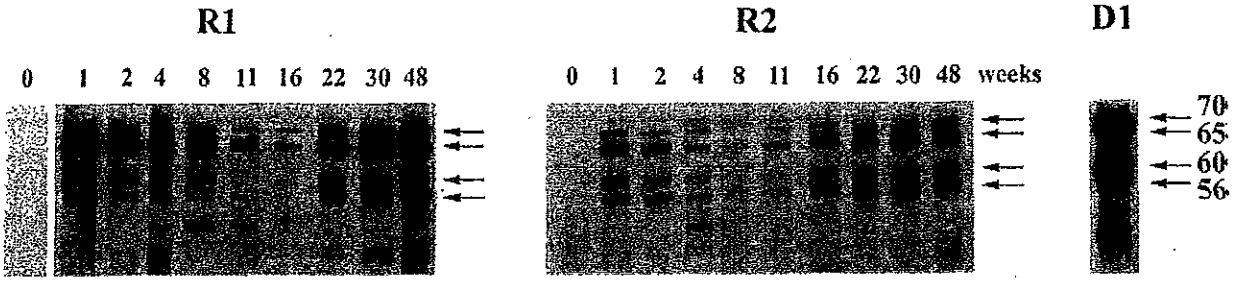


Fig. 1. Detection of SFV-specific antibodies by Western blot analysis. Monkey plasma samples, obtained on day of blood transfusion (Week 0) and at various weeks after transfusion (except Week 16, where serum was used), were incubated with immunoblot strips containing lysate prepared from SFV-2-infected *M. dunni* cells and proteins visualized as described under Materials and methods. A 5-second exposure of the autoradiogram is shown. The molecular masses of prominently visible, SFV-specific proteins, calculated from standard markers (MultiMark, Novex, San Diego, CA), are indicated in kilodaltons.

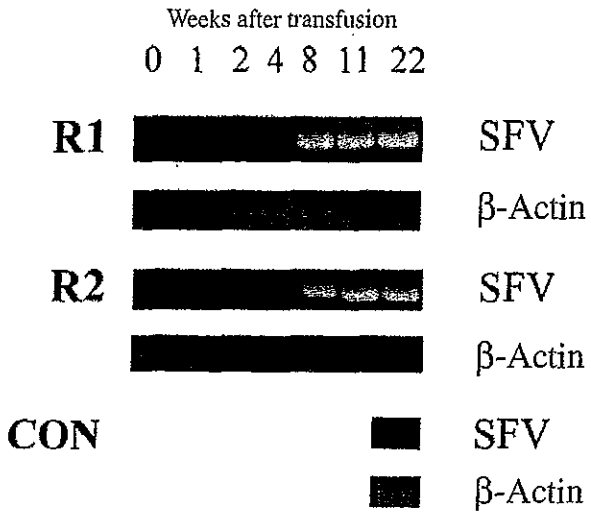


Fig. 2. Detection of SFV in monkey PBMNCs by PCR assay. SFV-specific primers were used to analyze PBMNC DNA as described under Materials and methods. DNA samples were prepared from PBMNCs that were obtained on the day of blood transfusion (Week 0) and at the indicated times after transfusion. PCR amplification with β-actin primers confirmed presence of DNA in the samples. CON = control.

the 22-week sample of R1 and R2 was confirmed by nucleotide sequence analysis. As shown in Fig. 3, the SFV sequences in R1 and R2 were identical to the SFV in D1. SFV-specific sequences were not detected in R3 and R4 at any time up to 30 weeks (the last tested time), including early time after transfusion, where passive antibodies were present (data not shown). The control animal was negative by PCR with SFV primers.

SFV isolation from monkey PBMNC

To determine whether the SFV sequences detected in R1 and R2 were associated with an infectious virus, monkey PBMNCs from Week 11 and Week 22 after transfusion were cocultured with *M. dunni* cells. The cultures were monitored for replicating SFV by the appearance of CPE in the cell monolayer and by RT production in cell-free supernatant. The RT results, shown in Fig. 4, indicate earlier virus isolation with the Week 11 sample from both R1 and R2, with culture termination due to extensive CPE at Day 14: in the case of the Week 22 sample, there was a slightly delayed kinetics of virus isolation with culture termination on Day 16. This difference in the kinetics of virus isolation was also evidenced by CPE detection in the cocultures, which was seen on Day 9 in the case of the Week 11 sample and on Day 11 with the Week 22 sample. The kinetics of virus isolation with PBMNC from the day of blood transfusion for D1 showed that CPE was seen on Day 11 with culture termination on Day 18. No virus was detected in PBMNC from R1 and R2 on the day of transfusion nor at any time from the control animal. There was no evidence of virus isolation from PBMNC of R3 and R4 at any time point tested including 1 year after blood transfusion, the last tested time; virus was isolated from D2 on the day of blood transfer (data not shown).

The identity of the viruses isolated in the coculture experiments with the Week 11 sample from R1 and R2 was confirmed by PCR amplification and nucleotide sequence analysis: the results indicated sequence identity with SFV-D1 (data not shown).

Characterization of donor monkeys

The selection of D1 and D2 as donors was initially based on the results of earlier infectivity studies, which demonstrated that the SFVs isolated from the PBMNC of D1 and D2 were distinct in their replication kinetics and CPE development: SFV-D1 had high replication and rapid CPE as compared with SFV-D2 (data not shown). To further investigate the differences in SFV transmission by blood transfusion with D1 and D2, the neutralizing antibody titer and PBMNC viral load were determined on stored samples from the animals. The results indicated a neutralizing antibody endpoint titer of 1:50 for D1 and 1:800 for D2. PBMNC viral load analysis indicated 32.4 IUPM for D1 and 3.8 IUPM for D2. Additionally, a retrospective analysis the CBC differential count indicated that the WBC count in D2 was about half of that in D1.

DISCUSSION

The identification of SFV-seropositive blood donors has raised safety concerns regarding SFV transmission by blood transfusion. A study analyzing recipients of blood components such as RBCs, filtered RBCs (WBC-reduced), PLTs, and fractionated plasma from one SFV-infected donor demonstrated absence of virus transmission,[16] however, PBMNC, which are known targets of SFV infection, were not examined and the results are limited by the sample size. Based on a theoretical risk the CDC has been counseling infected people not to donate blood.[16] To evaluate the potential risk of SFV transmission by blood and blood products, we have initially determined virus transmission by whole-blood transfusion in a monkey model. Blood was transferred from two donor animals that were naturally infected with SFVs that had distinct replication kinetics and nucleotide sequences. Interestingly, SFV transmission only occurred with D1: antibodies developed at 16 to 22 weeks and persisted approximately 1 year after transfusion (the last time tested); SFV sequences were detected by PCR at 8 weeks after transfusion, and infectious virus was isolated from PBMNC at Week 11 and Week 22. The lack of virus transmission with blood transfusion from D2 was unexpected because SFV has an

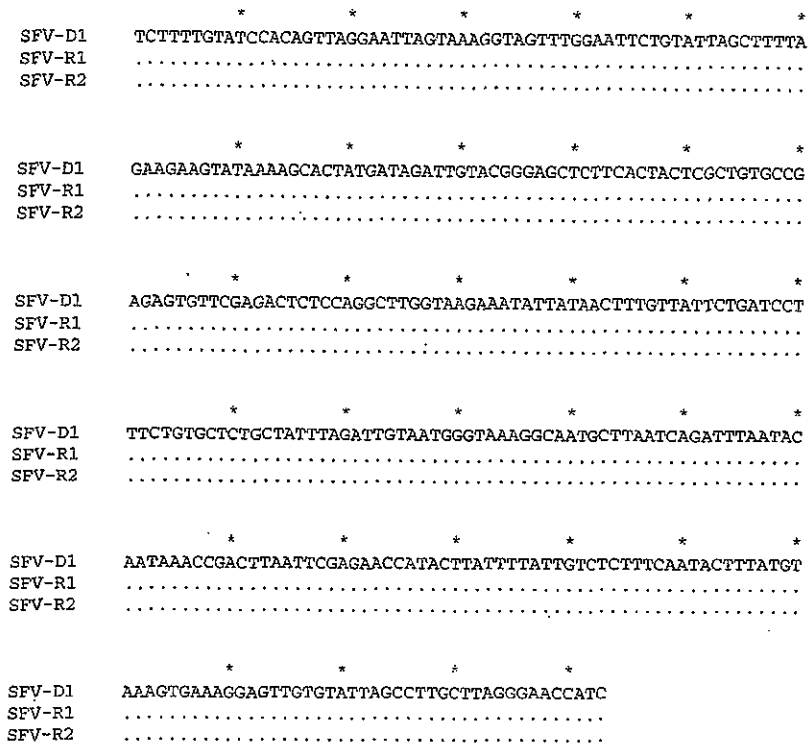


Fig. 3. Nucleotide sequence identification of SFV sequences in blood recipient monkeys. Nucleotide sequences of SFV in R1 and R2 (designated as SFV-R1 and SFV-R2, respectively) were determined from DNA fragments that were PCR-amplified from PBMNC at 22 weeks after transfusion (shown inFig. 2). Sequence comparison with SFV in the donor animal (SFV-D1) are shown: dots indicate base identity; asterisks indicate base count.

exceptionally broad host range and tissue tropism and is easily transmitted in NHPs, albeit via the saliva.[3] Different factors may contribute to retrovirus transmission such as virus load in the inoculum and fitness of the donor virus. Additionally, neutralizing antibodies have been shown to block SHIV infection of macaques.[25,26] Antibody analysis of D1 and D2 indicated a significantly higher neutralizing antibody endpoint titer in D2 compared to D1 (1:800 versus 1:50, respectively) suggesting that neutralizing antibodies may play a role in SFV transmission. Studies are under way to investigate the contribution of antibody titer in the failure of SFV transmission by D2. The results of these studies may provide insight regarding factors involved in SFV transmission and in assessing the risk of virus transmission by blood donors.

High viral load is an important determinant of virus transmission in HIV-1 infection.[27] In the case of SFV infection, the virus largely infects lymphocytes and monocytes,[3,14,15] and it is believed that virus is mostly cell-associated with no detectable virus in the plasma. Therefore, we initially determined the PBMNC viral load of D1 and D2: the results indicated that the IUPM was 32.4 and 3.8, respectively. Interestingly, this is similar to the PBMNC viral load reported in chronic infection with SIV in African green monkeys[28] and HIV-1 in humans.[29] Although the blood transfer volume was the same (10 mL), based on the CBC differential, it was found that D1 had twice the number of WBC as D2: thus approximately 29×10^6 PBMNC were transfused in case of D1 and 15×10^6 in case of D2 so that the approximate number of infected cells transferred by D1 was 940 cells and 57 by D2. Additional studies will be performed to determine whether the PBMNC viral load represents the total number of infected cells in blood and the contribution of plasma viral load, if any, in SFV transfusion transmission. It should be noted that virus fitness[30] may play an important role in virus transmission from D1 based upon in vitro studies indicating that SFV-D1 had earlier replication kinetics and more rapid CPE development than SFV-D2 (data not shown). The relationship between virus fitness and SFV transmission will be investigated to assess the risk of infection by blood transfusion.

Interestingly, virus isolation occurred with more rapid kinetics with the Week 11 PBMNC samples from R1 and R2 than with the Week 22 samples (Fig. 4). Furthermore, the kinetics of virus isolation from PBMC of chronically infected D1 was similar to that of Week 22 samples. This result suggests a higher PBMNC viral load early after infection, with a subsequent lower set point in long-term infection. To evaluate the kinetics of virus infection in vivo, longitudinal analysis of PBMNC viral load will be done on stored samples, including quantitative analysis by TaqMan PCR. Additionally, corresponding plasma samples will be tested for evidence of any SFV viremia. Analysis of PBMNC and plasma viral load may identify a high-risk window period of SFV transmission by blood transfusion. It is noteworthy that the apparent reduction in viral load in the Week 22 PBMNC samples coincided with the increase in SFV-specific antibodies (Table 1 and Fig. 1), thereby suggesting a potential role of neutralizing antibodies in reducing virus replication.

The consequences of cross-species transmission of retroviruses are unpredictable and may not be noticed for an extended period until there is a clinical outcome. This is most effectively evidenced by HIV-1, which was discovered in 1983 due to the AIDS epidemic[31] more than 50 years after the initial cross-species infection with SIV.[32,33] The lack of disease associated with SFV in any spe-

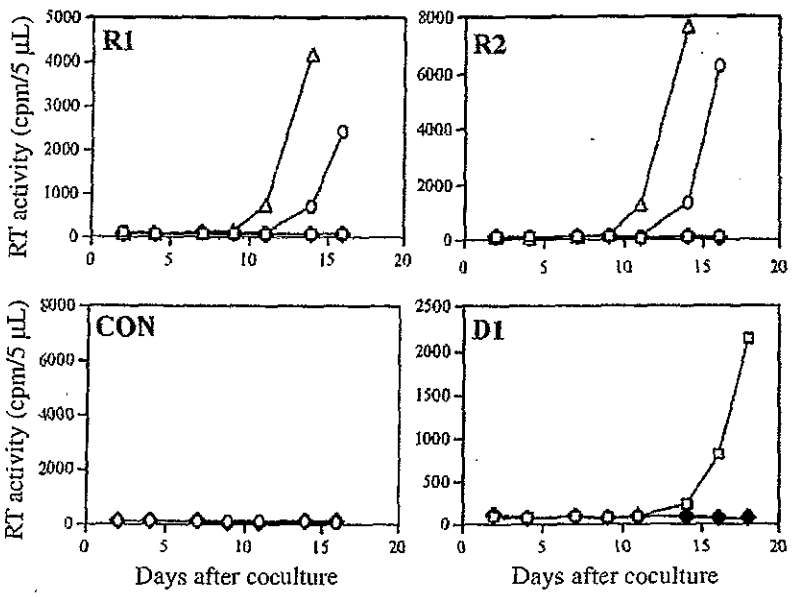


Fig. 4. SFV isolation from monkey PBMNC. PBMNC of R1 and R2, obtained on the day of blood transfer and at Weeks 11 and 22 after transfusion (2.0×10^6-2.3×10^6) were PHA-stimulated and cocultured with *M. dunni* cells, until the cultures were terminated due to extensive CPE. PBMNC from the control animal (CON: Week 22; 2.4×10^6) and donor D1 (day of blood transfusion; $<2.0 \times 10^6$) were PHA-stimulated, and cocultures set up as controls for PBMNC from negative and positive monkey, respectively. *M. dunni* cells without monkey PBMNC were included as cell culture control. Filtered supernatant was collected during the culture period and assayed for RT activity (mean ± standard deviation was calculated from two spots). Day of blood transfusion, □; Week 11 after transfusion, △ Week 22 after transfusion, ○; *M. dunni* control, ◆.

cies is an enigma,[3,4] especially since foamy viruses can be highly cytopathic in cells in vitro.[4] Due to the stable integration and long-term persistence of infectious viral sequences in the host genome, SFV might have an unexpected clinical outcome. Thus, similar to other retroviruses of public health impact, it is prudent take appropriate measures to avoid SFV exposure and infection.

The absence of known disease and lack of transmission in humans does not negate health concerns related to SFV infection in humans due to insufficient data. Demonstration of SFV infection by blood transfusion in a monkey model indicates potential risk for virus transmission in humans. The results support consideration of appropriate safeguards against exposure to SFV, or any other simian agent, through the human blood supply.

ACKNOWLEDGMENTS

We thank Lauren Davidson for the monkey blood transfusions, Raymond Olsen for monkey bleeds, and Philip Snoy and the veterinary staff at CBER for outstanding animal care. We thank Hira Nakhasi, Edward Tabor, and Jay Epstein for valuable scientific discussions and for providing helpful comments on the manuscript. We thank Elliot Cowan for facilitating contract support at SeraCare BioServices (previously BBI Biotech Research Laboratories, Gaithersburg, MD), for processing of monkey blood and Richard Herberling and Anthony Cook (Simian Diagnostic Laboratories) for helpful advice in establishing the virus neutralization assay.

REFERENCES

1. Apetrei C, Marx PA. Simian retroviral infections in human beings. Lancet 2004;364:137-8;author reply 139-40.
2. Aguzzi A. The foamy virus family: molecular biology, epidemiology and neuropathology. Biochim Biophys Acta 1993;1155:1-24.
3. Meiering CD, Linial ML. Historical perspective of foamy virus epidemiology and infection. Clin Microbiol Rev 2001;14:165-76.
4. Mergia A, Leung NJ, Blackwell J. Cell tropism of the simian foamy virus type 1 (SFV-1). J Med Primatol 1996;25:2-7.
5. Achong BG, Mansell PW, Epstein MA, Clifford P. An unusual virus in cultures from a human nasopharyngeal carcinoma. J Natl Cancer Inst 1971;46:299-307.
6. Schweizer M, Falcone V, Gange J, et al. Simian foamy virus isolated from an accidentally infected human individual. J Virol 1997;71:4821-4.
7. Schweizer M, Turek R, Hahn H, et al. Markers of foamy virus infections in monkeys, apes, and accidentally infected humans: appropriate testing fails to confirm suspected foamy virus prevalence in humans. AIDS Res Hum Retroviruses 1995;11:161-70.
8. Heneine W, Switzer WM, Sandstrom P, et al. Identification of a human population infected with simian foamy viruses. Nat Med 1998;4:403-7.
9. Brooks JI, Rud EW, Pilon RG, et al. Cross-species retroviral transmission from macaques to human beings. Lancet 2002;360:387-8.
10. Switzer WM, Bhullar V, Shanmugam V, et al. Frequent simian foamy virus infection in persons occupationally exposed to nonhuman primates. J Virol 2004;78:2780-9.
11. Heneine W, Schweizer M, Sandstrom P, Folks T. Human infection with foamy viruses. Curr Top Microbiol Immunol 2003;277:181-96.
12. Sandstrom PA, Phan KO, Switzer WM, et al. Simian foamy virus infection among zoo keepers. Lancet 2000;355:551-2.

13. Wolfe ND, Switzer WM, Carr JK, et al. Naturally acquired simian retrovirus infections in central African hunters. Lancet 2004;363:932-7.
14. von Laer D, Neumann-Haefelin D, Heeney JL, Schweizer M. Lymphocytes are the major reservoir for foamy viruses in peripheral blood. Virology 1996;221:240-4.
15. Callahan ME, Switzer WM, Matthews AL, et al. Persistent zoonotic infection of a human with simian foamy virus in the absence of an intact orf-2 accessory gene. J Virol 1999;73:9619-24.
16. Boneva RS, Grindon AJ, Orton SL, et al. Simian foamy virus infection in a blood donor. Transfusion 2002;42:886-91.
17. Delwart EL, Kalmin ND, Jones TS, et al. First report of human immunodeficiency virus transmission via an RNA-screened blood donation. Vox Sang 2004;86:171-7.
18. Donegan E, Lee H, Operskalski E, et al. Transfusion transmission of retroviruses: human T-lymphotropic virus types I and II compared with human immunodeficiency virus type 1. Transfusion 1994;34:478-83.
19. Heberling RL, Kalter SS. Rapid dot-immunobinding asay on nitrocellulose for viral antibodies. J Clin Microbiol 1986;23: 109-13.
20. Institute for Laboratory Animal Research NRC. Guide for the care and use of laboratory animals. Washington (DC): National Academy Press; 1996.
21. Khan AS, Sears JF, Muller J, et al. Sensitive assays for isolation and detection of simian foamy retroviruses. J Clin Microbiol 1999;37:2678-86.
22. Sears JF, Khan AS. Single-tube fluorescent product-enhanced reverse transcriptase assay with Ampliwax (STF-PERT) for retrovirus quantitation. J Virol Methods 2003;108:139-42.
23. Karber G. A contribution to the collective treatment of a pharmacological experimental series. Arch Exp Pathol Pharmakol 1931;162:480-3.
24. Hussain AI, Shanmugam V, Bhullar VB, et al. Screening for simian foamy virus infection by using a combined antigen Western blot assay: evidence for a wide distribution among Old World primates and identification of four new divergent viruses. Virology 2003;309:248-57.
25. Shibata R, Igarashi T, Haigwood N, et al. Neutralizing antibody directed against the HIV-1 envelope glycoprotein can completely block HIV-1/SIV chimeric virus infections of macaque monkeys. Nat Med 1999;5:204-10.
26. Nishimura Y, Igarashi T, Haigwood N, et al. Determination of a statistically valid neutralization titer in plasma that confers protection against simian-human immunodeficiency virus challenge following passive transfer of high-titered neutralizing antibodies. J Virol 2002;76:2123-30.
27. Campbell TB, Schneider K, Wrin T, et al. Relationship between in vitro human immunodeficiency virus type 1 replication rate and virus load in plasma. J Virol 2003; 77:12105-12.
28. Hartung S, Boller K, Cichutek K, et al. Quantitation of a lentivirus in its natural host: simian immunodeficiency virus in African green monkeys. J Virol 1992;66: 2143-9.
29. Ho DD, Moudgil T, Alam M. Quantitation of human immunodeficiency virus type 1 in the blood of infected persons. N Engl J Med 1989;321:1621-5.
30. Barbour JD, Grant RM. The role of viral fitness in HIV pathogenesis. Curr HIV/AIDS Rep 2005;2:29-34.
31. Karpas A. Human retroviruses in leukaemia and AIDS: reflections on their discovery, biology and epidemiology. Biol Rev 2004;79:911-33.
32. Korber B, Muldoon M, Theiler J, et al. Timing the ancestor of the HIV-1 pandemic strains. Science 2000;288: 1789-96.
33. Takebe Y, Kusagawa S, Motomura K. Molecular epidemiology of HIV: tracking AIDS pandemic. Pediatrics Int 2004;46:236-44.
34. Linial M. Why aren't foamy viruses pathogenic? Trends Microbiol 2000;8:284-9.

医薬品
医薬部外品　研究報告　調査報告書
化粧品

別紙 3-8

識別番号・報告回数		回	報告日 年　月　日	第一報入手日 2006 年 4 月 27 日	新医薬品等の区分 該当なし	総合機構処理欄
一般的名称			研究報告の公表状況	Development of an improved method of detection of infectious parvovirus B19 Wong, S. and Brown, K.E. J. Clin. Virol., 35, 407-413 (2006)	公表国 米国	
販売名（企業名）						

研究報告の概要

パルボウイルスB19は，ヒトに対してのみ病原性をもつパルボウイルスであるが，急性感染後は赤血球前駆細胞で高濃度まで複製する。その時点で多くの場合は無症候であるので，高度に汚染した血液であっても血液供給される。その上，非エンベロープであるパルボウイルスB19は現在の方法では容易に不活性化されない。この研究では様々な細胞株のB19感染に対する感受性を比較し，中和抗体と同様にウイルス感染を検出する様々な方法を評価した。
UT7/Epo-S1細胞株は，B19感染に最も高い感受性があることが判明した。そしてこの株では，間接免疫蛍光法により容易にB19カプシドタンパクが染色された。最も高感度の感染症分析はRNA転写を検出するRT-PCRあるいは定量的RT-PCR法であった。また，RNA転写を検出するハイスループット分析法が開発され，血漿プールに含まれた感染ウイルスを高力価で検出できた。さらに本分析法を確認する手段として血清で中和抗体を検出した。その結果，抗B19抗体を含む血清でプレインキュベートしたB19ウイルス感染UT7/Epo-S1細胞株では，RNA転写が顕著に減少していた。ここで紹介された分析法は，現存するものより多くの利点を示している。

使用上の注意記載状況・その他参考事項等

BYL-2006-0225

報告企業の意見	今後の対応
弊社血漿分画製剤の製造工程における各段階におけるウイルス除去効果は，パルボウイルス B19 のモデルとしてブタパルボウイルスを用いて確認されている。ヒトトランスフェリンでは 5.9log 以上，ヒトアルブミンでは 6.8log 以上，またヒト免疫グロブリンでは 9.3log までウイルス除去が可能である。したがって，弊社の血漿分画製剤では，ウイルス伝播のリスクは極めて低い。	現時点で新たな安全対策上の措置を講じる必要は無いと考える。引き続き関連情報の収集に努める。

(12)

ELSEVIER

Journal of Clinical Virology 35 (2006) 407–413

JOURNAL OF CLINICAL VIROLOGY

www.elsevier.com/locate/jcv

Development of an improved method of detection of infectious parvovirus B19

Susan Wong, Kevin E. Brown *

Hematology Branch, National Heart, Lung and Blood Institute, Bethesda, MD, USA

Received 14 November 2005; received in revised form 23 December 2005; accepted 23 December 2005

Abstract

Background: Parvovirus B19, the only known pathogenic human parvovirus is the aetiologic agent of erythema infectiosum, transient aplastic crisis, pure red cell aplasia, and hydrops fetalis. Transmission is either by respiratory secretions or, as it can be present at high titre in plasma, by blood and blood products. B19 is only cultured with difficulty in vitro, and there is no readily available assay for detecting B19 infectivity or neutralizing antibodies.

Objectives: In this study, we evaluated different methods to detect viral infection for the purpose of developing automated methods for large-scale testing of viral infectivity, development of neutralizing antibody and viral inactivation assays.

Study design: Different cell lines were evaluated for their ability to support B19 infection and assays tested for sensitivity and ease of performing. A high-throughput assay was validated by determining infectious virus in blood pools and for determining neutralizing antibody in sera.

Results: B19 protein production was detected by immunofluorescence (IF) staining and increased viral DNA production by dot blot hybridization and quantitative PCR. The detection of RNA transcripts by RT-PCR assay and quantitative RT-PCR (qRT-PCR) was used as an indirect marker for infection. Of the cell lines tested, the subclone UT7/Epo-S1 showed the greatest sensitivity to B19 infection, with detection of viral transcripts by qRT-PCR the preferred assay. The assays were validated by experiments to determine the infectious titre of sera from acutely infected humans, to evaluate the presence of infectious virus in human donor plasma pools and to measure neutralizing antibodies.

Keywords: Parvovirus B19; Erythrovirus infections; Neutralizing antibody

1. Introduction

Parvovirus B19 is the only known parvovirus pathogenic to humans. It is associated with a number of diseases including erythema infectiosum ("fifth disease") in children, arthropathy commonly seen in women, transient aplastic crisis in individuals with high red cell turnover, pure red cell aplasia in immunocompromised patients, and hydrops fetalis following infection during pregnancy (Young and Brown, 2004).

B19 is highly erythrotropic and replicates to high titre in erythroid progenitor cells. In healthy individuals, at the height of the transient viraemia, viral titres as high as 10^{13} genome equivalents (ge)/mL are detectable. Individuals are often asymptomatic at this time, and highly viraemic blood donations do enter the blood supply. In addition, as the virus has only a small (5600 nucleotide) DNA genome, and is non-enveloped, the virus is relatively heat resistant (Schwarz et al., 1992) and not removed by solvent/detergent methods normally used to inactivate virus (Mortimer et al., 1983b; Sayers, 1994). Depending on the sensitivity of the assay, B19 DNA can be detected in between 0.11% and 0.003% of blood donor samples (Jordan et al., 1998; McOmish et al., 1993; Mortimer et al., 1983b; Tsujimura et al., 1995; Wakamatsu et al., 1999), and transmission of B19 infection to recipients of both blood

* Correspondence to: Virus Reference Department, Centre for Infections, Health Protection Agency, 61 Colindale Avenue, London, NW9 5HT, UK. Tel: +44 20 8327 6023; fax: +44 20 8327 6019.

E-mail address: Kevin.Brown@HPA.org.uk (K.E. Brown).

1386-6532/$ – see front matter © 2006 Elsevier B.V. All rights reserved.
doi:10.1016/j.jcv.2005.12.008

and blood products has been frequently documented (Azzi et al., 1999). Attempting to reduce the risk of B19 infection has become a major concern for blood and blood product suppliers in Europe and America (Brown et al., 2001), with many countries now advocating screening of plasma pools to remove samples containing high B19 viral titres.

However, B19 DNA appears to be very stable and low levels of B19 DNA can persist in serum and a range of tissues for months following acute infection (Soderlund-Venermo et al., 2002), and detection of viral DNA does not equate necessarily with active viral infection. In addition, assays for detecting infectious virus are extremely limited. Although B19 has been shown to replicate in vitro in primary erythroid progenitor cells from bone marrow (Mortimer et al., 1983a), peripheral blood (Ozawa et al., 1986) and fetal liver (Brown et al., 1991; Yaegashi et al., 1989), very few cell lines have been found to be permissive for B19 infection, and even in these, viral replication is inefficient. The first cell line described to be permissive for B19 infection was an erythropoietin (Epo)-dependent subclone of UT7, a megakaryoblastoid cell line (Shimomura et al., 1992). Since then, a limited number of additional cell lines have been described, including KU812Ep6, an erythroleukaemic cell line (Miyagawa et al., 1999), and JK-1 cells (Takahashi et al., 1993). To date no comparative studies of the differences in susceptibility/sensitivity and permissivity amongst these cell lines have been published.

A number of different methods have been suggested for detecting parvovirus B19. Currently, methods for identifying active clinical B19 infections include detection of B19 nucleic acid testing by direct dot blot hybridization (Anderson et al., 1985) or PCR (Cassinotti et al., 1993; Clewley, 1992), and more recently RT-PCR for RNA transcripts (Wong et al., 2003). Similar methods have been used for detecting infection in cells or cell lines, but little has been published on the relative sensitivity of the different methods.

In this study we compared the susceptibility/sensitivity of various cell lines to B19 infection and evaluated different methods to detect viral infection. In addition, we established a high-throughput method for detection of B19 infection and validated the assay by using it to detect infectious virus in plasma pools and neutralizing antibodies in serum samples.

2. Materials and methods

2.1. Cell lines

Cell lines were obtained from American Tissue Type Collection (ATCC, Manassas, VA, USA) and maintained in Dulbecco's modified Eagle medium (DMEM) plus 10% fetal calf serum (FCS), penicillin, streptomycin and glutamine (P/S/G; Gibco/Invitrogen, Carlsbad, CA, USA) at 37 °C with 5% CO_2 unless otherwise stated. UT7/Epo (Shimomura et al., 1992) were maintained in RPMI 1640 with 10% FCS and 5 U/mL of Epo (Amgen, Thousand Oaks, CA, USA). UT7/Epo-S1, a subclone of UT7/Epo and a gift from Dr Sagamura (Morita et al., 2001) were maintained in Iscove's modified Dulbecco's media (IMDM) plus 10% FCS, and 2 U Epo/mL. KU812Ep6, a gift from Dr Miyagawa (Miyagawa et al., 1999) were maintained in RPMI 1640, 10% FCS and 6 U Epo/mL K-562 and JK-1 cells (Deutsche Sammlung von Mikroorganismen und Zellkulturen (DSMZ), Braunschweig, Germany) were maintained in RPMI 1640, with 10% FCS.

2.2. B19, serum and plasma sources

Plasma and serum samples containing high-titre infectious B19 was obtained from several sources. J35 was obtained from a sickle-cell patient in aplastic crisis, and previously determined to be negative for both B19 IgM and IgG (data not shown). Additional plasma samples were obtained from normal donors at the time of blood donation, and provided by Mei-Ying Yu at CBER, FDA and Aris Lazo at V.I. Technologies, Inc. The WHO standard sample (Saldanha et al., 2002) was obtained from National Institutes of Biological Standards and Control (NIBSC), South Mimms, UK.

Plasma pools containing high-titre B19 were a kind gift of Matthias Gessner of Baxter. Serum samples from laboratory donors and healthy blood donors obtained as part of the National Heart, Lung, and Blood Institute (NHLBI) Retrovirus Epidemiology Blood Donor Study, NHLBI repository, and previously tested for antibody to parvovirus B19 (Brown et al., 2004) were used in the neutralization assay.

2.3. Comparison of infectivity of different cell types

The infection assay was as previously described (Nguyen et al., 2002). Briefly, on the day before infection, cells were split 1:2 with appropriate media to induce cell division. On the day of infection, cells were seeded at 2×10^5 cells/100 μL, an equal volume of virus dilution was added and the infection was allowed to incubate at 4 °C for 2 h. After the incubation, 800 μL of appropriate media was added to the infection bringing the volume to 1 mL. The cells were transferred to a 24-well plate and incubated at 37 °C and harvested for analysis on Days 1, 2, 3, or 6.

2.4. Immunofluorescence (IF) assay for B19 capsid proteins

Cells were evaluated for B19 protein production by IF staining. Approximately 5×10^4 cells were collected onto glass slides by cytocentrifugation at 1500 rpm for 8 min and fixed in methanol–acetone (1:1) at −20 °C. Viral capsid proteins were detected by mouse anti-B19 monoclonal antibody, 521-5D (a gift from Larry Anderson, CDC, Atlanta, GA, USA) followed by goat anti-mouse IgG FITC (Zhi et al., 2004).

2.5. B19 DNA

DNA direct hybridization was used to quantitate the B19 copy number as previously described (Brown et al., 1994). Briefly, plasmid pYT103 was diluted to give a range of DNA concentrations (0, 0.1, 1, 10, and 100 pg/μl). Plasmid dilutions, serum samples or extracted DNA (10 μl) were added to 200 μl 0.333 M NaOH and incubated at room temperature to denature the DNA. The samples were then applied to a nylon membrane (0.45 μm pore, Nytran Plus, Schleicher and Schuell, Keene, NH, USA) using a dot blot manifold apparatus (96-well, Schleicher and Schuell), the membrane washed in 6× SSC, and the membrane baked at 80 °C for an hour in a vacuum oven. The membranes were hybridized with a ^{32}P random-primed probe of the complete B19 coding region (*Eco*RI digest of pYT103) in Hybrisol (Serologicals Corporation; 42 °C overnight). The membranes were washed with 2× SSC; 0.1% SDS at room temperature and 0.1× SSC; 0.1% SDS for 20 min at 55 °C, and then exposed to either a "phosphor" screen (Molecular Dynamics, GE Healthcare) or X-ray film.

2.6. Quantitative PCR

DNA was extracted from cells and supernatant using the QIAmp DNA mini Kit (Qiagen, Valencia, CA, USA), and quantitated by qPCR using the QuantiTect Probe PCR kit (Qiagen), using primers in the capsid region of the virus. Specifically the primers and probe used were B19-Cap-F (5′-TACCTGTCTGGATTGCAAAGC-3′; 0.4 μM) and B19-Cap-R (5′-GATGGGTTTTCTAGGGGATTATC-3′; 0.4 μM) and 0.2:M B19-Cap-Probe probe (6-FAM-ATG GTG GGA AAG TGA TGA TGA ATT TGC TA-Black Hole Quencher). Quantitation of the number of genome copies was estimated by comparison to a standard curve obtained from serial dilutions of the pYT103, and confirmed by testing the NIBSC standard (Saldanha et al., 2002).

2.7. RT-PCR

As previously described, an RT-PCR assay was used to look for spliced viral transcripts as a marker for infection (Nguyen et al., 2002). Briefly, cells were harvested and total RNA extracted using 200 μL RNA STAT-60. Contaminating DNA was removed using RQ1 DNAse (Promega) incubation for 15 min at room temperature and RNA was reverse transcribed by initially incubating RNA with random primers and reverse transcriptase, Superscript II (Invitrogen), prior to PCR amplication with primers B19-9 and B19-1. To increase detection of spliced products from the RT-PCR reactions, products were resolved on a 2.5% NuSieve agarose gel and southern hybridization was performed using probe labeled with alkaline phosphatase (CDP-Star AlkPhos labeling kit, Amersham).

2.8. Quantitative RT-PCR

RNA transcripts were quantitated by real-time RT-PCR designed to amplify products in the capsid and NS regions using the QuantiTect Probe RT-PCR kit (Qiagen). The QuantiTect Probe RT-PCR master mix and QuantiTect RT mix was combined with 0.4 μM of the amplification primers (NS primers 5′-GTTTTATGGGCCGCCAAGTA-3′ and 5′-ATCCCAGACCACCAAGCTTTT-3′; capsid primers 5′-CCTGGGCAAGTTAGCGTAC-3′ and 5′-ATGAATCCTTGCAGCACTGTCA-3′), and 0.2 μM probe (NS probe FAM 6′-CCATTGCTAAAAGTGTTCCA-BHQ1; capsid probe FAM-TATGTTGGGCCTGGCAA-TAMRA). After an initial activation step of 15 min at 95 °C, 45 cycles of 15 s at 94 °C and 60 s at 60 °C were performed. Quantitation of the number of transcripts was by estimating the cDNA copy number by comparison of a standard curve of serial dilutions of pYT103 as described for the quantitative PCR.

To confirm extraction of RNA, and to normalize the number of transcripts per cell, quantitative RT-PCR (qRT-PCR) was performed using the same amplification conditions, but with primers β-actin F (5′-GGCACCC-AGCACAATGAAG-3′), β-actin R (5′-GCCGATCCACA-CGGAGTACT-3′) and actin probe (5′JOE-TCAAGA-TCATTGCTCCTCCTGAGCGC-3′BHQ). An actin standard curve was obtained from serial dilutions of a plasmid containing an extended region of the actin coding sequence.

2.9. High-throughput qRT-PCR

To develop a high-throughput method for detecting RNA transcripts, RNA was extracted from cells using GeneStrips™ (RNAture, Irvine, CA, USA) according to the manufacturer's protocols. mRNA extracted using this method was converted to cDNA using MMLV-RT (Invitrogen, Carlsbad, CA, USA) following manufacturer's protocol scaled up to a 50 μL reaction volume. B19 RNA transcript production was determined by qRT-PCR as described above.

In some experiments the infection volume was scaled down to 100 μL and incubated in 96-well plates.

2.10. Detection of infectious virus in plasma pools

Plasma pools of 2000 donors that contained B19 DNA by PCR B19 were tested for their ability to infect UT7/Epo-S1 cells. Infection was as described (100 μL infection volume) and RNA was extracted with RNAture Genestrips and analyzed by qRT-PCR. The number of B19 DNA copies in the original plasma pools was determined by quantitative PCR.

2.11. Neutralizing antibody detection

Detection of neutralizing antibodies was assayed by qRT-PCR. Serum or plasma from donors was incubated with serial dilutions of high-titre B19 containing serum for 1 h prior to

infection with UT7/Epo-S1 cells as described for infections (100 μL in a 96-well plate). RNA was extracted from cells using the RNAture Genestrips and analyzed by qRT-PCR.

3. Results

3.1. Comparison of sensitivity and permissiveness in haematopoietic cell lines

The majority of the haematopoietic lines tested (HL-60, HEL, KG-1, KG-1a, K-562, U-937) were negative by both IF for capsid protein and detection of spliced transcripts indicating that the cells were non-permissive. Only UT7/Epo cells, KU812Ep6, the UT7/Epo-S1 subclone and JK-1 cells showed evidence of B19 infection with the UT7/Epo-S1 cells having the greatest sensitivity. However, by IF, the number of positive cells was always low, with <1% positive staining for KU812Ep6, UT7/Epo and JK-1 cells, but approximately 15% positive staining for UT7/Epo-S1 cells. This greater sensitivity was confirmed by detection of transcripts in UT7/Epo-S1 cells at 10^4 ge/infection of the high-titre serum, 3 logs lower than that detected in the other cell lines. Subsequent studies were all done with the UT7-Epo-S1 cells.

3.2. Comparison of sensitivity of different methods to detect infectious B19

IF staining for B19 capsid protein production detected infected cells consistently at 10^{-2} to 10^{-3} dilutions of high-titre serum (>10^{12} ge/mL), but the number of positive cells was low (15–1%). Determining viral DNA production by direct hybridization of viral DNA by dot blot was limited to detection above 10^9 ge/infection, in part because only 10 μL of sample was analyzed from a 1 mL infection. In time course experiments, when infecting with high concentrations of virus (10^9 ge/mL) spliced transcripts could be detected on the first day, rising on the third day. When samples were tested on Day 3, the most sensitive methods were those detecting RNA transcripts, either by conventional or qRT-PCR assay or detecting the increase in viral DNA production by qPCR (Table 1).

Table 1
Comparison of the different sensitivities of assays used to determine B19 infectivity

Detection method	Sensitivity, genome equivalents (ge)
Protein: IF	~10^8
DNA: dot blot hybridization	10^9
DNA: quantitative PCR	10^4
RNA: RT-PCR	10^4
RNA: quantitative RT-PCR	10^4

UT7/Epo-S1 cells were infected with dilutions of B19, and cells assayed on Day 3. Results are the minimum amount of virus added to a 1 mL cell culture to detect infectivity.

Table 2
Comparison of the number of infectious units of B19 to the viral DNA in different serum or plasma samples

Viral stock	Genome equivalents ((ge)/mL × 10^{12})	Infectious (units/mL)	ge/infectious units
J35	33.2 ± 2.0	10^8	3.3×10^5
VS2	10.2 ± 2.9	10^9	1.0×10^4
V1	2.0 ± 0.3	10^8	2.0×10^4
V2	2.9 ± 0.2	10^8	2.9×10^4
V3	0.1 ± 0.1	2×10^7	5×10^3
CBER STD	1.0	10^8	1.0×10^4

Viral DNA measured by qPCR. UT7/Epo-S1 cells were infected with serial dilutions of virus, on Day 3 RNA was extracted with RNA STAT60 and B19 transcripts detected by RT-PCR.

3.3. Comparison of the infectivity of different serum samples with viral copy number

Serial dilutions of different viral sera stock were used to infect UT7/Epo-S1 cells, and the infectious titre determined by the endpoint of detection using RT-PCR (Table 2). The viral genome equivalents in each sample was determined for each sample by qPCR and confirmed by dot blot analysis and the ratio of viral DNA (ge) were compared to infectious units. The ratio of genome equivalents were relatively high compared to infectious units, with ratios ranging from 2×10^5 ge in the J35 stock to 5×10^3 ge per infectious unit in the CBER standard.

3.4. Comparison of RNA extraction methods

Cell lysates were directly incubated in RNAture "Genestrips", washed off, and cDNA synthesized in situ, prior to qPCR. In direct comparion tests RNA extracted from scaled down 0.1 mL cultures with RNAture Genestrips showed comparable sensitivity to 1 mL cultures extracted with RNA STAT-60 (Table 3).

Table 3
Equivalent sensitivity of detection of infectious virus using two different methods of RNA extraction and B19 transcript detection

Virus dilution	Standard method		High-throughput method	
	Day 0	Day 3	Day 0	Day 3
10^{-5}	–	+++	0	27,345
10^{-6}	–	+++	0	1328
10^{-7}	–	+++	0	231
10^{-8}	–	–	0	1
10^{-9}	–	–	0	0

A plasma stock (V1) was serially diluted in culture medium and used to infect UT7/Epo-S1 cells. Standard method, RNA was extracted from a 1 mL culture using RNA STAT-60 and transcripts detected by RT-PCR. +++, bands easily detected by ethidium bromide staining and with an alkaline phosphatase-labeled specifc probe; –, bands not detected; and high-throughput method, RNA was extracted from a 0.1 mL culture using RNAtureGenestrips and transcripts detected by NS qRT-PCR. Quantitations are given as ge/μL of RT reaction volume.

Table 4
Detection of infectious B19 in plasma pools

Sample #	Stock (ge)/mL × 10^{12}	RT-PCR result	VP qRT-PCR (ge/infection) × 10^8	NS qRT-PCR (ge/infection) × 10^8
Pool #1	1.8 ± 0.4	+++	2.1 ± 0.9	2.2 ± 1.4
Pool #2	3.2 ± 0.1	+++	4.6 ± 0.8	3.7 ± 0.9
Pool #3	0.5 ± 0.1	+++	2.8 ± 0.3	2.1 ± 0.4
Pool #4	0.5 ± 0.04	+++	2.6 ± 0.9	2.4 ± 0.5
Pool #5	0.005 ± 0.0005	+	0.0023 ± 0.001	0.0036 ± 0.002
Pool #6	1.1 ± 0.3	+++	3.0 ± 0.2	1.4 ± 0.05
No virus	0	–	0	0

The amount of B19 in six plasma pools (previously known to contain B19) was determined by qPCR and the presence of infectious virus was determined by infection of UT7/Epo-S1 cells and detection of RNA transcripts after by either RT-PCR or using RNAture Genestrips and qRT-PCR amplifying the capsid (VP) and nonstructural (NS) regions.

3.5. Detection of infectious virus in pooled blood products

To test the sensitivity of our high-throughput assay and to test its potential application for screening plasma pools for infectious virus we examined six plasma pools previously identified as containing high-titre B19 DNA. qPCR analysis confirmed that all samples contained B19 DNA, with five of the six samples containing high B19 titres with >10^{11} ge/mL. The sixth sample had ~5 × 10^9 ge/mL. However, after infection of UT7/Epo-S1 cells, RNA transcripts could be detected by both RT-PCR and qRT-PCR, indicating that all the pools contained infectious B19 (Table 4).

3.6. Neutralization

As further validation of the high-throughput assay the method was used to detect B19 neutralizing antibodies in six sera of known B19 antibody status. After incubation with serial dilutions of each serum with virus for 2 h, the antibody/virus complex was allowed to infect UT7/Epo-S1 cells in a microtitre plate, RNA extracted at Day 3 RNAture Genestrips, and qRT-PCR performed. No block of infection was detected in the two sera that were B19 IgG negative (Table 5). In contrast, at the highest concentration of virus, there was a marked reduction in the number of viral transcripts with all four sera. If a reduction of viral transcripts by >90% is considered the endpoint, then in two sera the neutralizing titre was >10^5, and in the other 10^3 and 10^4, respectively.

4. Discussion

Although the erythroid tropism and inhibition of erythroid colony formation was demonstrated in 1983 (Mortimer et al., 1983a) and replication in vitro infection of bone marrow was demonstrated in 1986 (Ozawa et al., 1986), there is still no readily available method for culturing parvovirus B19 in the laboratory, limiting both the virus availability and the ability to develop assays to determine B19 infectivity. Similarly there are no readily available methods for detecting neutralizing antibodies in patient samples, or for testing viral inactivation procedures for blood and blood products.

A number of cell lines have been described that support B19 infection, and a number of infectivity assays have been described, based on these cell lines. Specifically, infection and neutralization assays based on UT7/Epo cells (Bostic et al., 1999), KU812Ep6 (Blumel et al., 2002; Bonvicini et al., 2004; Miyagawa et al., 1999; Saito et al., 2003) and UT7/Epo-S1 (Prikhod'ko et al., 2005) cells have all been described. More recently, cells that are not fully permissive for B19 infection have also been evaluated (Caillet-Fauquet et al., 2004) However, there have been no attempts to compare the different cell types or sensitivity of the different methods. In addition, many of the methodologies are very labour intensive, and/or require reading of IF, and are therefore not readily automated or applicable to testing large numbers of samples.

In our study, IF was the least sensitive of the methods, with apart from UT7/Epo-S1 cells, generally less than 1% of cells being positive even after inoculation with high-titre virus.

Table 5
Neutralizing antibody assay using quantitative RT-PCR in the B19 NS region

Serum sample	4	5	44	45	A	B
IgG	+	+	–	+	+	–
Serum alone	0	0	9	0	0	8
B19 + 10^{-3} serum	189	0	5244	0	0	7393
B19 + 10^{-4} serum	30,215	3	14,423	50	34	5801
B19 + 10^{-5} serum	40,657	64	68,165	423	12,674	79,557
B19 alone	30,027	20,709	24,234	19,666	11,448	20,676
Cells only	0	0	0	0	1	2

Normal donor serum was preincubated with dilutions of high-titre B19 plasma (>10^{12} ge/mL) and used to infect UT7/Epo-S1 cells. A representation of quantitative data obtained is shown and given in ge/μL of RT reaction volume and normalized against the qPCR obtained for β-actin.

This percentage of positive cells was lower than published in the literature: Miyagawa reported KU812Ep6 cells as having about 30% of the cells positive for B19 infection (Miyagawa et al., 1999), and Morita reported that 40% of the UT7/Epo-S1 stained positive for B19 infection (Morita et al., 2001). Some of these discrepancies may be due to the amount of virus used for the infection (at higher titre we can observe that 30% of cells are positive), differences in culture techniques, and the specificity of the antibody used (Mab 521-5d used in these studies is specific for capsid conformational epitopes). However, as reading IF slides is not readily automated we did not spend more time optimizing the method.

Assays based on RT-PCR were the most sensitive assays, and in contrast to DNA-based assays were not confounded by input viral genomes. As with the IF assay, they also confirmed that the UT7/Epo-S1 cells were the most sensitive cell line, and using these cells, we could detect infectious virus with inoculums of $\sim 10^4$ genome copies. This is also in keeping with other published results that suggest the ratio of infectious virus:genome copies is 1:10,000 (Bonvicini et al., 2004; Miyagawa et al., 1999), not dissimilar to that of other *Parvoviridae* (Tattersall and Cotmore, 1988).

Due to the concerns of B19 contamination in blood and blood product, there is currently great interest in not only developing methods to detect infectious virus, but also to evaluate methods of viral inactivation/removal. Due to the difficulty in working with B19, many inactivation studies have been undertaken using a surrogate parvovirus, normally porcine parvovirus. However, when comparisons have been undertaken, porcine parvovirus and B19 have different properties as far as heat inactivation (Blumel, 2004) and pH stability (Boschetti et al., 2004), suggesting where possible the model virus should be studied. Although we, with colleagues, have previously described infections assays based on UT7/Epo cells by detection of spliced transcripts (Bostic et al., 1999; Lazo et al., 2002) or quantitative PCR (Prikhod'ko et al., 2005), we believe that the combination or RNA extraction process with quantitative RT-PCR is the easiest method for detecting B19 infection under a wide range of different clinical and experimental conditions.

Finally, the detection of neutralizing antibodies for parvovirus B19 continues to be challenging. Although methylcellulose-based assays were originally described, they are insensitive, and require large amounts of infectious virus, and are quite labour-intensive both to set up and to read. In contrast, the assay described here, requires small amount of B19 virus, and can be readily set up in a microtitre plate format. In some patients, especially immunocompromised patients, who have low levels of DNA in serum or tissues in the presence of B19 IgG, the decision as to whether treatment with IVIG would be beneficial can be difficult. Measurement of neutralizing antibodies in these circumstances would be helpful. In addition, such assays will be critical in determining the response to B19 immunization when the B19 vaccine becomes available.

5. Conclusions

This assay can be used to determine the infectious titre of parvovirus B19 in a number of different settings. In addition, the ability to automate many of the steps in the assay may allow this assay to be used more widely than is currently available.

Acknowledgments

This research was supported by the Intramural Research Program of the National Heart, Lung and Blood Institute, NIH.

References

Anderson MJ, Jones SE, Minson AC. Diagnosis of human parvovirus infection by dot-blot hybridization using cloned viral DNA. J Med Virol 1985;15:163–72.

Azzi A, Morfini M, Mannucci PM. The transfusion-associated transmission of parvovirus B19. Transfus Med Rev 1999;13:194–204.

Blumel J. Cell culture-based assay of parvovirus B19 and the relevance of animal model viruses. Dev Biol (Basel) 2004;118:107–12.

Blumel J, Schmidt I, Willkommen H, Lower J. Inactivation of parvovirus B19 during pasteurization of human serum albumin. Transfusion 2002;42:1011–8.

Bonvicini F, Gallinella G, Cricca M, et al. Molecular testing for detection of in vitro infectivity of plasma pools contaminated with B19 virus. J Med Virol 2004;74:272–6.

Boschetti N, Niederhauser I, Kempf C, Stuhler A, Lower J, Blumel J. Different susceptibility of B19 virus and mice minute virus to low pH treatment. Transfusion 2004;44:1079–86.

Bostic JR, Brown KE, Young NS, Koenig S. Quantitative analysis of neutralizing immune responses to human parvovirus B19 using a novel reverse transcriptase-polymerase chain reaction-based assay. J Infect Dis 1999;179:619–26.

Brown KE, Hibbs JR, Gallinella G, et al. Resistance to parvovirus B19 infection due to lack of virus receptor (erythrocyte P antigen). N Engl J Med 1994;330:1192–6.

Brown KE, Liu Z, Gallinella G, Wong S, Mills IP, O'Sullivan MG. Simian parvovirus infection: A potential zoonosis. J Infect Dis 2004;190:1900–7.

Brown KE, Mori J, Cohen BJ, Field AM. In vitro propagation of parvovirus B19 in primary foetal liver culture. J Gen Virol 1991;72:741–5.

Brown KE, Young NS, Alving BM, Barbosa LH. Parvovirus B19: Implications for transfusion medicine. Summary of a workshop. Transfusion 2001;41:130–5.

Caillet-Fauquet P, Di Giambattista M, Draps ML, Hougardy V, de Launoit Y, Laub R. An assay for parvovirus B19 neutralizing antibodies based on human hepatocarcinoma cell lines. Transfusion 2004;44:1340–3.

Cassinotti P, Weitz M, Siegl G. Human parvovirus B19 infections: Routine diagnosis by a new nested polymerase chain reaction assay. J Med Virol 1993;40:228–34.

Clewley JP. Polymerase chain reaction diagnosis of human parvovirus B19. In: Becker Y, Darai G, editors. Diagnosis of human viruses by polymerase chain reaction technology. New York: Springer-Verlag; 1992. p. 285–96.

Jordan J, Tiangco B, Kiss J, Koch W. Human parvovirus B19: Prevalence of viral DNA in volunteer blood donors and clinical outcomes of transfusion recipients. Vox Sang 1998;75:97–102.

Lazo A, Tassello J, Ohagen A, Aytay S, Wong S, Brown K. INACTINE technology inactivates clinical isolates of parvovirus B19 in RBC

concentrates as demonstrated by PCR and infectivity assays. Blood 2002:100 [abstract 1094, ref type: abstract].

McOmish F, Yap PL, Jordan A, Hart H, Cohen BJ, Simmonds P. Detection of parvovirus B19 in donated blood: A model system for screening by polymerase chain reaction. J Clin Microbiol 1993;31:323–8.

Miyagawa E, Yoshida T, Takahashi H, et al. Infection of the erythroid cell line, KU812Ep6 with human parvovirus B19 and its application to titration of B19 infectivity. J Virol Methods 1999;83:45–54.

Morita E, Tada K, Chisaka H, et al. Human parvovirus B19 induces cell cycle arrest at G(2) phase with accumulation of mitotic cyclins. J Virol 2001;75:7555–63.

Mortimer PP, Humphries RK, Moore JG, Purcell RH, Young NS. A human parvovirus-like virus inhibits haematopoietic colony formation in vitro. Nature 1983a;302:426–9.

Mortimer PP, Luban NL, Kelleher JF, Cohen BJ. Transmission of serum parvovirus-like virus by clotting-factor concentrates. Lancet 1983b;ii:482–4.

Nguyen QT, Wong S, Heegaard ED, Brown KE. Identification and characterization of a second novel human erythrovirus variant, A6. Virology 2002;301:374–80.

Ozawa K, Kurtzman G, Young N. Replication of the B19 parvovirus in human bone marrow cell cultures. Science 1986;233:883–6.

Prikhod'ko GG, Vasilyeva I, Reyes H, et al. Evaluation of a new LightCycler reverse transcription-polymerase chain reaction infectivity assay for detection of human parvovirus B19 in dry-heat inactivation studies. Transfusion 2005;45:1011–9.

Saito T, Munakata Y, Fu Y, et al. Evaluation of anti-parvovirus B19 activity in sera by assay using quantitative polymerase chain reaction. J Virol Methods 2003;107:81–7.

Saldanha J, Lelie N, Yu MW, Heath A. Establishment of the first World Health Organization International Standard for human parvovirus B19 DNA nucleic acid amplification techniques. Vox Sang 2002;82:24–31.

Sayers MH. Transfusion-transmitted viral infections other than hepatitis and human immunodeficiency virus infection. Cytomegalovirus, Epstein–Barr virus, human herpesvirus 6, and human parvovirus B19. Arch Pathol Lab Med 1994;118:346–9.

Schwarz TF, Serke S, von Brunn A, et al. Heat stability of parvovirus B19: Kinetics of inactivation. Int J Med Microbiol Virol Parasitol Infect Dis 1992;277:219–23.

Shimomura S, Komatsu N, Frickhofen N, Anderson S, Kajigaya S, Young NS. First continuous propagation of B19 parvovirus in a cell line. Blood 1992;79:18–24.

Soderlund-Venermo M, Hokynar K, Nieminen J, Rautakorpi H, Hedman K. Persistence of human parvovirus B19 in human tissues. Pathol Biol (Paris) 2002;50:307–16.

Takahashi T, Ozawa K, Takahashi K, et al. DNA replication of parvovirus B 19 in a human erythroid leukemia cell line (JK-1) in vitro. Arch Virol 1993;131:201–8.

Tattersall P, Cotmore SF. The nature of parvoviruses. In: Pattison JR, editor. Parvoviruses and human disease. Florida: CRC Press; 1988. p. 5–41.

Tsujimura M, Matsushita K, Shiraki H, Sato H, Okochi K, Maeda Y. Human parvovirus B19 infection in blood donors. Vox Sang 1995;69:206–12.

Wakamatsu C, Takakura F, Kojima E, et al. Screening of blood donors for human parvovirus B19 and characterization of the results. Vox Sang 1999;76:14–21.

Wong S, Young NS, Brown KE. Prevalence of parvovirus B19 in liver tissue: No association with fulminant hepatitis or hepatitis-associated aplastic anemia. J Infect Dis 2003;187:1581–6.

Yaegashi N, Shiraishi H,. Takeshita T, Nakamura M, Yajima A, Sugamura K. Propagation of human parvovirus B19 in primary culture of erythroid lineage cells derived from fetal liver. J Virol 1989;63: 2422–6.

Young NS, Brown KE. Parvovirus B19. N Engl J Med 2004;350:586–97.

Zhi N, Zadori Z, Brown KE, Tijssen P. Construction and sequencing of an infectious clone of the human parvovirus B19. Virology 2004;318:142–52.

医薬品 研究報告 調査報告書

識別番号・報告回数			報告日	第一報入手日	新医薬品等の区分	機構処理欄
				2006. 6. 20	該当なし	

一般的名称	(製造承認書に記載なし)	研究報告の公表状況	Doyle S, Corcoran A. J Infect Dis. 2006 Jul 15;194(2):154-8. Epub 2006 Jun 9.	公表国 アイルランド
販売名(企業名)	合成血「日赤」(日本赤十字社) 照射合成血「日赤」(日本赤十字社)			

研究報告の概要

○パルボウイルスB19暴露に対する抗体陰性および陽性の人における免疫反応
ウイルスが混入した血液製剤を投与された後のパルボウイルスB19に対する免疫反応に関する情報はほとんど得られていない。最近の研究で、B19DNAを含む(1.6x10^{6}IU/mL)プール血漿の輸血後、B19抗体陽性の患者のB19IgG抗体のレベルが19-39 IU/mLから50-100 IU/mLまで上昇して再感染を防いだことを発見した。B19抗体陰性の患者における1.6-2.2 x 10^{8}IU/mLのB19DNAの存在は、プール血漿のIgGレベル59.5 IU/mLではB19の伝播とそれに続くセロコンバージョンを防ぐのは不十分であることがわかった。これらのデータは血液製剤の安全性評価の進歩につながるだろう。

報告企業の意見	今後の対応
B19DNAを含む(1.6x10^{6}IU/mL)プール血漿の輸血後、B19抗体陽性の患者では抗体価が上昇して再感染を防いだが、陰性の患者における1.6-2.2 x 10^{8}IU/mLのB19DNAの存在は、プール血漿のIgGレベル59.5IU/mLではB19の伝播とセロコンバージョンを防ぐのは不十分であることがわかったとの報告である。	今後も引き続き、ヒトパルボウイルスB19に関する新たな知見及び情報の収集に努める。日本赤十字社では、以前よりRHA法によるB19抗原検査を導入、ウイルス量の多い血液を排除している。今後は検査方法の改善によりさらなる感度向上を目指すこととしている。

使用上の注意記載状況・その他参考事項等

合成血「日赤」
照射合成血「日赤」

血液を介するウイルス、
細菌、原虫等の感染
vCJD等の伝播のリスク

BRIEF REPORT

The Immune Response to Parvovirus B19 Exposure in Previously Seronegative and Seropositive Individuals

Sean Doyle and Amanda Corcoran*

Department of Biology and National Institute for Cellular Biotechnology, National University of Ireland, Maynooth, County Kildare, Ireland

Little information is available on the immune response to parvovirus B19 after the administration of contaminated blood products. In the present study, we found that levels of B19 IgG in B19-seropositive recipients protect against re-infection and, after transfusion with pooled plasma containing B19 DNA (1.6×10^8 IU/mL), increase from 19–39 IU/mL to 50–100 IU/mL. We found that, in the presence of 1.6–2.2 $\times 10^8$ IU of B19 DNA/mL in B19-seronegative recipients, a pooled-plasma B19 IgG level of 59.5 IU/mL is insufficient to prevent B19 transmission and subsequent seroconversion. These data should lead to improvements in the assessment of blood-product safety.

Parvovirus B19 can cause severe disease in immunocompromised individuals, and B19 infection during pregnancy can lead to fetal mortality. B19 infection is transmitted either via respiratory secretions or via administration of contaminated blood or blood products. The latter mode of transmission is especially problematic because of the high resilience of B19 to many of the treatments used in plasma processing, such as solvent-detergent treatment, lyophilization, and high temperatures [1], and also because of the extremely high levels of viremia in acutely infected, and often asymptomatic, individuals (>10^{12} B19 DNA genome equivalents [GE]/mL or IU/mL) [2].

Significant efforts to minimize the B19 viral load in blood products commenced in the late 1990s because of the advent of robust DNA extraction and B19 polymerase chain reaction methodologies in addition to cases of B19 seroconversion in healthy volunteers who received contaminated plasma as part of a postmarketing surveillance study [3]. Most manufacturers now undertake minipool B19 nucleic acid testing to reduce plasma-pool levels of B19 DNA to <10^4 IU/mL, to conform with US Food and Drug Administration (FDA) proposals (available at: http://www.fda.gov/). Standardization of B19 DNA and IgG quantitation, as well as the establishment of validated serological assay systems, has also contributed to improvements in blood-product screening paradigms. The regulatory requirement that levels of B19 DNA in anti-D antibody preparations be <10^4 IU/mL [4] further illustrates the actions taken by regulatory agencies to effectively improve blood-product safety.

In the future, because of enhanced screening protocols, B19 transmission after the administration of blood products should become a less frequent event. However, heightened awareness of B19 has resulted in the emergence of relevant information regarding the infectious dose of B19 and the role played by B19 IgG in attenuating transmission. Koenigbauer et al. [5] reported a case of B19 infection in a 36-year-old woman that resulted from administration of a solvent/detergent-treated pooled plasma that was subsequently recalled by the American Red Cross after high levels (10^7–10^8 GE/mL B19 DNA) of B19 DNA were detected by the manufacturer. Blumel et al. [6] detailed 2 cases of B19 infection resulting from the administration of B19 IgG^- plasma protein–complex concentrates: 1 individual received 180 mL of heat-treated concentrate containing 8.6×10^6 GE of B19 DNA/mL (1.5×10^9 GE total), and the other received 996 mL of material containing 4×10^3 GE of B19 DNA/mL (3.9×10^6 GE total). The transmission of B19 by a factor VIII concentrate (free of B19 IgG) has been documented in a case in which seroconversion occurred as a result of infusion of 2×10^4 IU of B19 DNA (1.3×10^3 IU/mL) [7]. Solvent/detergent-treated plasma (Plas+SD) has also been identified, subsequent to a postmarketing surveillance study of this product, as the source of B19 infection that occurred in 18 individuals [3, 8]. It was concluded that B19 IgG in pooled plasma (64.7 ± 17.5 IU of B19 IgG/mL; [9]) was not protective in the presence of high B19 viral titers (10^7–10^8 GE/mL) and that plasma lots containing low viral titers ($10^{0.5}$–$10^{3.5}$ GE/mL) did not cause B19 infection in plasma recipients. However, detailed serological analysis of this event has not been forthcoming, and the significance that the data have for wider issues of

Received 13 January 2006; accepted 7 March 2006; electronically published 9 June 2006.
Potential conflicts of interest: none reported.
Financial support: Quality of Life and Management of Living Resources Program, Commission of the European Communities (project QLK2-CT-2001-00877 ["Human Parvovirus Infection: Towards Improved Understanding, Diagnosis and Therapy"]); Irish Health Research Board.

* Present affiliation: Biotrin, Mount Merrion, County Dublin, Ireland.
Reprints or correspondence: Dr. Sean Doyle, Dept. of Biology and National Institute for Cellular Biotechnology, National University of Ireland, Maynooth, Co. Kildare, Ireland (sean.doyle@nuim.ie).

The Journal of Infectious Diseases 2006; 194:154–8

0022-1899/2006/19402-0004$15.00

B19 infectivity and immunity merits consideration. In the present article, we describe the serological analysis of specimens obtained from 10 individuals who participated in the postmarketing study [3, 8]; this analysis extends our knowledge of the immune response to B19 exposure.

Materials and methods. As part of a postmarketing study, 100 adult volunteers, previously determined to be B19 IgG$^+$ by use of an *Escherichia coli*–based EIA to detect B19 IgG, were each transfused with 1 unit (200 mL) of pooled plasma (Plas+SD) [3, 8, 10]. Paired plasma specimens (blinded) were obtained pretransfusion and 1 month posttransfusion from 10 of the volunteers.

The 20 plasma specimens were analyzed for both B19 IgM and B19 IgG reactivity against capsid (conformational) VP2 (VP2-N), by use of FDA-approved EIAs (Biotrin). B19 IgG levels were quantified using the World Health Organization B19 IgG International Standard (93/724) [11]. Furthermore, B19 IgG reactivity against conformational (N) and linear (D) epitopes on VP1 (VP1-N and VP1-D, respectively) and to linear VP2 (VP2-D) was analyzed as described elsewhere [12]. The subsequent classification of pooled-plasma recipients into groups I, II, and III, as well as the details of plasma pools used for transfusion, is shown in table 1.

Results. Figure 1*A* shows that specimens from groups I and II contained no VP2-specific IgM reactivity, whereas specimens from group III exhibited high levels of B19 IgM reactivity posttransfusion, thereby confirming acute B19 infection in this cohort. Analysis of the B19 IgG reactivity of individual plasma specimens was performed both pre- and posttransfusion, and 3 specimens (01002, 01052, and 01098) of 10 exhibited reactivity against VP2-N pretransfusion, with the range of B19 IgG in these specimens being 19–39 IU/mL (figure 1*B*, group I); there was a subsequent increase in the level of B19 IgG reactivity posttransfusion, which resulted in 2 of 3 specimens (both transfused with plasma pool PS3 [table 1]) exhibiting B19 IgG levels >100 IU/mL and the third specimen exhibiting an increase to 50 IU/mL.

A further 3 specimens (01023, 01053, and 01055), 2 of which were from individuals transfused with plasma pool PS2A (table 1), were seronegative for antibodies against VP2-N (B19 IgG <3 IU/mL), both pre- and posttransfusion (figure 1*B*, group II).

The remaining 4 paired pretransfusion specimens tested contained no detectable B19 IgG against VP2-N; however, the corresponding paired posttransfusion specimens exhibited evidence of B19 seroconversion and exhibited high levels of reactivity against VP2-N epitopes (figure 1*B*, group III); this reactivity corresponded to B19 IgG levels >100 IU/mL in 2 of 4 of the specimens, whereas the remaining 2 specimens contained lower levels of B19 IgG, equivalent to 50 and 78 IU/mL, respectively. For each specimen, the pattern of reactivity against VP1-N epitopes was identical to that exhibited against VP2-N, whereby IgG specific for VP1-N was increased posttransfusion in group I and was also evident only posttransfusion in group III (figure 1*C*).

When specimens were analyzed for reactivity against VP1-D epitopes, 2 specimens (01002 and 01098) of 3 from group I did not exhibit significant pretransfusion IgG reactivity; however, these 2 specimens did display significant posttransfusion antibody reactivity (mean ± SD IgG index value, 3.5 ± 1.7 [IgG index value >1.1 is reactive]) (figure 1*D*). The remaining specimen (01052) was seronegative for VP1-D IgG, both pre- and posttransfusion. Group II specimens were unreactive against VP1-D. All group III specimens were seronegative for B19 VP1-D IgG pretransfusion; posttransfusion, however, all had high levels of antibody reactivity against VP1-D epitopes (mean ± SD IgG index value, 3.9 ± 0.96).

B19 IgG reactivity was observed only against VP2-D epitopes in group III specimens, with a mean ± SD VP2-D IgG index value of 4.5 ± 1.8 (figure 1*E*). It should be noted that, although posttransfusion group I specimens exhibited an increase in levels of B19 IgG against VP2-N epitopes (figure 1*B*), they had no increase in antibody reactivity to VP2-D epitopes.

Discussion. The present study demonstrates that, in B19-seropositive recipients transfused with plasma containing high levels of B19 DNA (1.6×10^8 IU/mL), levels of parvovirus B19 IgG against VP1-N and VP2-N epitopes and against linear

Table 1. Classification of pooled-plasma recipients, according to B19 IgG reactivity against conformational epitopes on B19 VP2.

Group no., pooled-plasma–recipient code no.	Plasma pool transfused	B19 DNA level
Group I		
01002	PS3	1.6×10^8 IU/mL
01052	NA	NA
01098	PS3	1.6×10^8 IU/mL
Group II		
01023	PS2A	$10^{3.5}$ GE/mL
01053	PS2A	$10^{3.5}$ GE/mL
01055	NA	NA
Group III		
01005	PS1	2.2×10^8 IU/mL
01048	PS1	2.2×10^8 IU/mL
01057	PS1	2.2×10^8 IU/mL
01069	PS3	1.6×10^8 IU/mL

NOTE. Plasma pools PS1 and PS3 contained 59.5 and 72.0 IU of B19 IgG/mL, respectively [9]. The level of B19 DNA in plasma pool PS2A was provided by A. Lazo. Group I and II recipients remained symptom free, whereas group III recipients experienced mild fever and malaise, after transfusion. Group I, recipients who were seropositive before transfusion ($n = 3$); group II, recipients who were seronegative both before and after transfusion ($n = 3$); group III, recipients who were seronegative before transfusion and seropositive after transfusion ($n = 4$); GE, genome equivalent; NA, not available.

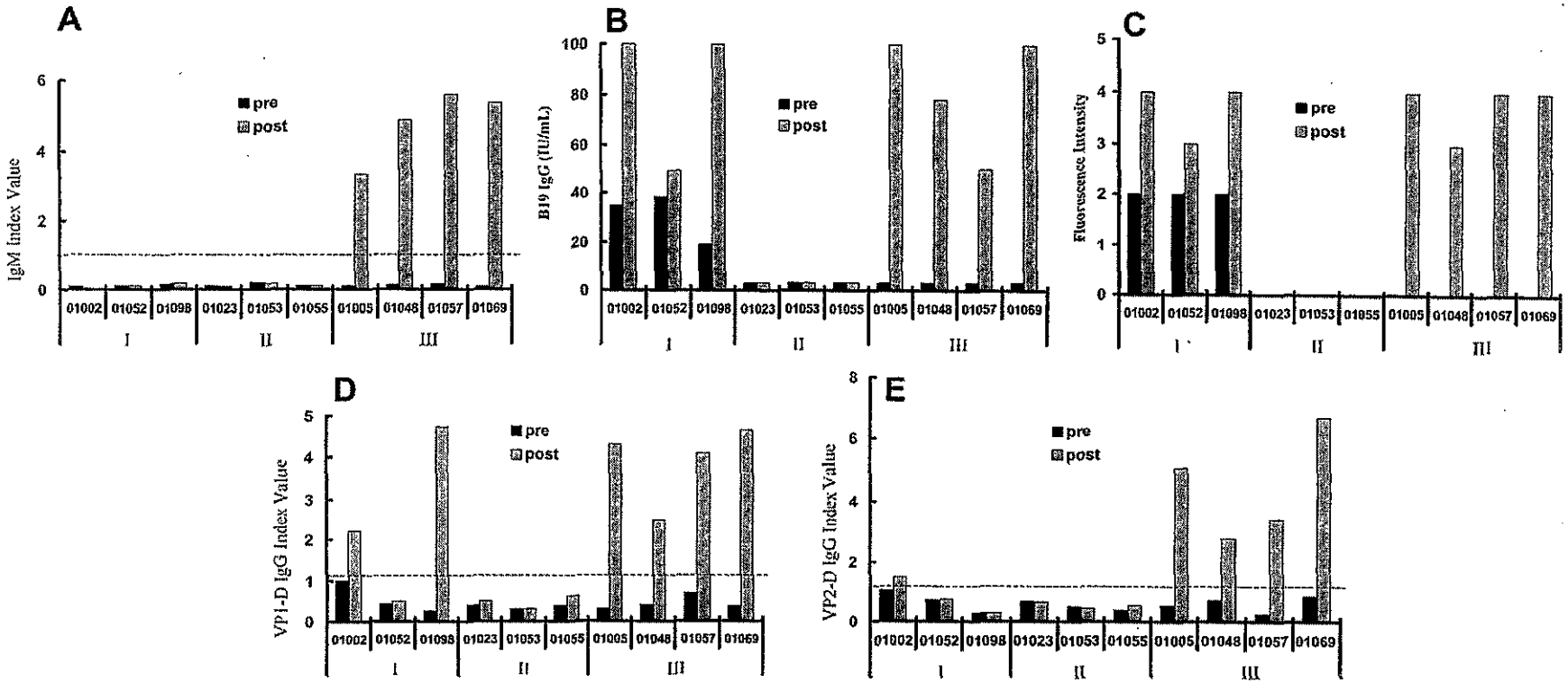


Figure 1. *A*, B19 IgM reactivity against conformational VP2, in pooled-plasma recipients (before and after transfusion). Reactivity is measured as IgM index value (reactivity >1.1 is reactive *[dashed line]*). *B*, B19 IgG reactivity against capsid (conformational) VP2 (VP2-N), as determined by EIA and expressed as IU/mL, in pooled-plasma recipients. *C*, B19 IgG reactivity against conformational epitopes on VP1 (VP1-N), in pooled-plasma recipients. Reactivity is assessed using an immunofluorescence assay and is graded, according to the manufacturer's instructions on a scale of 1–4, depending on the extent of fluorescence. *D*, B19 IgG reactivity against linear VP1 (VP1-D), in pooled-plasma recipients (before and after transfusion). *E*, B19 IgG reactivity against linear VP2 (VP2-D), in pooled-plasma recipients. *D* and *E*, Reactivity measured as IgG index value (index value is specimen:cutoff OD ratio; reactivity >1.1 is reactive *[dashed line]*).

epitopes on the unique region of VP1 increase dramatically. Moreover, it also proposes that the levels of B19 IgG in pooled-plasma products protect against infection in B19-seronegative recipients when only low levels of B19 DNA (i.e., $<10^{3.5}$ GE/mL) are present. Finally, we have shown that, in the presence of $1.6–2.2 \times 10^8$ IU of B19 DNA/mL, B19 IgG levels of 59.5 (plasma pool PS1) and 72.0 IU/mL (plasma pool PS3), respectively, are insufficient to prevent B19 transmission to B19-seronegative recipients (group III) and subsequent seroconversion.

Group I recipients were seropositive for B19 IgG before transfusion with pooled plasma. The level of IgG specific for VP2-N increased to >100 IU/mL in 2 recipients after transfusion with plasma pool PS3; however, the observed increase in the remaining recipient (01052) was lower (50 IU/mL). This subsequent increase in IgG response was mirrored by the increased reactivity against VP1-N that was observed posttransfusion, whereby the increase in fluorescence exhibited by the specimen from recipient 01052 was less than that for the others in group I. It is relevant that, because of the presence of high-titer B19 DNA, blood products lacking B19-specific antibodies were most at risk of transmitting B19 infection and that, despite high levels of B19 DNA, recipients with preexisting B19 IgG (or who were the administered blood products containing B19 IgG) were not infected [13]. Plentz et al. [14] have also confirmed that the presence of B19 IgG in either the recipients of the blood products or in the administered material offers protection against B19 DNA (at concentrations of $<6 \times 10^2–2.2 \times 10^6$ GE/mL) present in therapeutic products, to the extent that no individual ($n = 14$) receiving a B19-contaminated blood product showed symptoms of acute B19 infection. The results of the present study demonstrate that, in a healthy immunocompetent individual (recipient 01098), a B19 IgG level of 19 IU/mL confers protection against the development of symptoms of B19 infection when that individual is reexposed to the virus. To our knowledge, the present study is the first to demonstrate that there is a specific level of B19 IgG that protects against reinfection. The postexposure B19 IgG profile will also contribute to avoidance of reinfection.

Although all recipients in group I had either lost or never developed antibody reactivity against VP1 or VP2 epitopes before transfusion, 2 of them subsequently displayed strong IgG responses against linear epitopes on the VP1-unique region only and not against VP2-D. This observation is in accordance with the work of Soderlund et al. [15] and significantly strengthens our hypothesis that VP1-specific B-cell memory is maintained only with respect to linear epitopes of the unique region of VP1, as well as with respect to VP1-N/VP2-N epitopes [12]. Recipient 01052 in group I exhibited the lowest increase in B19 IgG reactivity after transfusion and was seronegative for antibody reactivity against VP1-D both before and after transfusion, possibly as a result of infusion with plasma containing a B19 viral load lower than that required for reactivation of the memory response.

Group II recipients all remained seronegative after receipt of pooled plasma. Although information was not available on which plasma pool was transfused into recipient 01055, both recipient 01023 and recipient 01053 were transfused with plasma pool PS2A, which contained $10^{3.5}$ B19 GE/mL [3]. Given that the mean level of B19 IgG observed in pooled plasma is 64.7 ± 17.5 IU/mL [9], it is clear that B19 IgG within this range appears to be protective against infection of seronegative recipients when the B19 viral load is $\leq 10^{3.5}$ B19 GE/mL.

Group III recipients who underwent B19 seroconversion after transfusion exhibited both strong VP2-specific IgM reactivity and significant levels of B19 IgG against VP2-D epitopes. The latter result is in accordance with previously published findings that production of antibody directed against VP2-D epitopes occurs shortly after exposure to B19 [15].

Traditionally, plasma-product manufacturers have relied on the presence of high levels of B19 IgG in pooled-plasma products alone to indicate product safety [2]. The data presented in the present study reinforce the strategy of identifying and removing high-titer B19 plasma donations from plasma pools, given that 4 of 7 recipients seroconverted because of the presence of B19 DNA in solvent/detergent-treated pooled human plasma. Although many companies have introduced minipool screening to address this problem, such screening is not presently mandatory, despite the fact that it is usually high-risk populations (e.g., pregnant women and immunocompromised patients) who are administered such products. This regulatory ambiguity is likely to change in coming years, as improved product-safety profiles are demanded by consumers.

In summary, the present study has provided new data relevant to the B19 IgG level necessary to confer protection after reexposure to the virus, as well as to the B19 IgG level that, in pooled-plasma products, may prevent infection of seronegative recipients.

References

1. Santagostino E, Mannucci PM, Gringeri A, et al. Transmission of parvovirus B19 by coagulation factor concentrates exposed to 100 degrees C heat after lyophilization. Transfusion 1997; 37:517–22.
2. Saldanha J, Lelie N, Yu MW, Heath A, B19 Collaborative Study Group. Establishment of the first World Health Organization International Standard for human parvovirus B19 DNA nucleic acid amplification techniques. Vox Sang 2002; 82:24–31.
3. Brown KE, Young NS, Alving BM, Barbosa LH. Parvovirus B19: implications for transfusion medicine. Summary of a workshop. Transfusion 2001; 41:130–5.
4. European Pharmacopoeia. European pharmacopoeia monograph of human anti-D immunoglobulin [document 01:0557]. Strasbourg, France: European Pharmacopoeia, 2004.
5. Koenigbauer UF, Eastlund T, Day JW. Clinical illness due to parvovirus

B19 infection after infusion of solvent/detergent-treated pooled plasma. Transfusion 2000; 40:1203–6.
6. Blumel J, Schmidt I, Effenberger W, et al. Parvovirus B19 transmission by heat-treated clotting factor concentrates. Transfusion 2002; 42: 1473–81.
7. Wu CG, Mason B, Jong J, et al. Parvovirus B19 transmission by a high-purity factor VIII concentrate. Transfusion 2005; 45:1003–10.
8. Davenport R, Geohas G, Cohen S, et al. Phase IV study of Plas+®SD: hepatitis A (HAV) and parvovirus B19 (B19) safety results [abstract 1942]. Blood 2000; 96(Suppl):451A.
9. Daly P, Corcoran A, Mahon BP, Doyle S. High-sensitivity PCR detection of parvovirus B19 in plasma. J Clin Microbiol 2002; 40:1958–62.
10. Laub R, Strengers P. Parvoviruses and blood products. Pathol Biol (Paris) 2002; 50:339–48.
11. Ferguson M, Walker D, Cohen B. Report of a collaborative study to establish the international standard for parvovirus B19 serum IgG. Biologicals 1997; 25:283–8.
12. Corcoran A, Mahon BP, Doyle S. B cell memory is directed toward conformational epitopes of parvovirus B19 capsid proteins and the unique region of VP1. J Infect Dis 2004; 189:1873–80.
13. Jordan J, Tiangco B, Kiss J, Koch W. Human parvovirus B19: prevalence of viral DNA in volunteer blood donors and clinical outcomes of transfusion recipients. Vox Sang 1998; 75:97–102.
14. Plentz A, Hahn J, Knoll A, Holler E, Jilg W, Modrow S. Exposure of hematologic patients to parvovirus B19 as a contaminant of blood cell preparations and blood products. Transfusion 2005; 45:1811–5.
15. Soderlund M, Brown CS, Spaan WJ, Hedman L, Hedman K. Epitope type-specific IgG responses to capsid proteins VP1 and VP2 of human parvovirus B19. J Infect Dis 1995; 172:1431–6.

別紙様式第2-1
番号16

医薬品
医薬部外品　　研究報告　調査報告書
化粧品

識別番号・報告回数			報告日	第一報入手日 2006年7月10日	新医薬品等の区分 該当なし	厚生労働省処理欄

一般的名称	①②ポリエチレングリコール処理人免疫グロブリン ③人免疫グロブリン	研究報告の公表状況	Thrombosis and Haemostasis 2004; 92: 838-845	公表国 ドイツ
販売名（企業名）	①献血ヴェノグロブリン-IH ヨシトミ（ベネシス） ②ヴェノグロブリン-IH（ベネシス） ③グロブリン-Wf（ベネシス）			

研究報告の概要

ヒトパルボウイルスB19 DNAは、しばしば血漿由来凝固因子製剤に検出される。加えてB19感染の伝播が観察され、製造工程中に日常的に行われるウイルスの不活化/除去の工程にもかかわらず、感染性ウイルスが存在していることを示している。最近、ヒトパルボウイルスB19の分類が3つの異なる遺伝型に分かれることが確認された。これまで、凝固因子製剤の遺伝子型2による汚染の情報はない。このため、我々はPCRにより、遺伝子型1及び2について、202の異なる凝固因子製剤のロットを調査した。最近3年間に投与された13の異なる製品の181のロットについて分析を行い、1980年代初めまで使用されたウイルス不活化処理のされていなかった21ロット（8製品）と比較した。遺伝子型1 DNAが、現在投与されているロットの77/181（42.5%）に、以前に使用されたロットの17/21（81%）に検出された。遺伝子型2 DNAは、5/202（2.5%）に見出され、その5ロット全てが、遺伝子型1 DNAにも汚染されていた。遺伝子型2 DNAが見出された5ロットは、血液凝固第VIII因子製剤の5ロットで、現在使用されているロットで2ロット、1980年代初めまで使用されていたロットで3ロットが含まれていた。
DNA配列分析は、PCRで2重に陽性であった製剤は、典型的な遺伝子型1と遺伝子型2のDNAを含んでいることを示していた。遺伝子型2は遺伝子型1と類似の疾患スペクトラムを起こすように見えることから、現在プール血漿に広く適用されている遺伝子型1に加えてNATでの遺伝子型2の同時検出によって、血液製剤の安全性のレベルを引き上げることができるであろう。

使用上の注意記載状況・その他参考事項等

代表として献血ヴェノグロブリン-IH ヨシトミの記載を示す。
2. 重要な基本的注意
(1)略
1) 血漿分画製剤の現在の製造工程では、ヒトパルボウイルスB19等のウイルスを完全に不活化・除去することが困難であるため、本剤の投与によりその感染の可能性を否定できないので、投与後の経過を十分に観察すること。
3. 略
4. 略
5. 略
6. 妊婦，産婦，授乳婦等への投与妊婦又は妊娠している可能性のある婦人には、治療上の有益性が危険性を上回ると判断される場合にのみ投与すること。〔妊娠中の投与に関する安全性は確立していない。本剤の投与によりヒトパルボウイルスB19の感染の可能性を否定できない。感染した場合には胎児への障害（流産，胎児水腫，胎児死亡）が起こる可能性がある。〕

報告企業の意見	今後の対応
ヒトパルボウイルスB19の変異型である遺伝型2が血液凝固第VIII因子製剤から検出された報告である。 弊社が最終製剤の試験に用いているキットは医学生物学研究所製「スマイテストパルボウイルスB19遺伝子定性キット」であり、このキットは、パルボウイルスB19遺伝型2についても検出可能であることを確認している。万一、原料血漿にパルボウイルスB19遺伝型2が混入したとしても、CPVをモデルウイルスとしたウイルスバリデーション試験成績およびパルボウイルスB19を用いた不活化・除去試験結果から、本剤の製造工程において十分に不活化・除去されると考えている。	本報告は本剤の安全性に影響を与えないと考えるので、特段の措置はとらない。

Contamination of coagulation factor concentrates with human parvovirus B19 genotype 1 and 2

Beate Schneider[1], Maria Becker[1], Hans-Hermann Brackmann[2], Anna Maria Eis-Hübinger[1]

[1]Institute of Medical Microbiology and Immunology and [2]Haemophilia Centre, Institute of Experimental Haematology and Transfusion Medicine; University of Bonn, Germany

Summary

Human parvovirus B19 (B19) DNA has frequently been detected in plasma-derived coagulation factor concentrates. Furthermore, transmission of B19 infection was observed, indicating presence of the infectious virus despite routine viral inactivation/removal procedures during the manufacturing process. Recently, human parvovirus DNA isolates, variant from B19, have been identified resulting in classification of B19 virus into three distinct genotypes, with all viruses previously classified as B19 belonging to genotype 1. So far, there is no information available on contamination of clotting factor concentrates with genotype 2. Therefore, we analysed 202 different factor concentrate lots for genotype 1 and 2 DNA by PCR. Analysis of one hundred eighty-one lots representing 13 different products, administered over the last three years, was compared to 21 lots (8 products) used until the early 1980s which had not been treated by viral inactivation procedures. Genotype 1 DNA was detected in 77/181 (42.5%) currently administered lots, and 17/21 (81%) previously used lots. The level of genotype 1 DNA contamination was similar in currently and previously administered concentrates. Genotype 2 DNA was found in 5/202 (2.5%) lots, all of which were co-contaminated with genotype 1 DNA. DNA sequence analysis showed that the PCR-double positive concentrates contained typical genotype 1 and genotype 2 DNA. Because genotype 2 appears to cause a similar spectrum of diseases as genotype 1, simultaneous detection of genotype 2 by nucleic acid amplification testing (NAT), now widely applied to plasma pools for genotype 1, would give an added level of safety to blood products.

Keywords

Human parvovirus, genotype 2, coagulation factor concentrates

Thromb Haemost 2004; 92: 838–45

Introduction

Human parvovirus B19 (B19) DNA has frequently been detected in plasma-derived coagulation factor concentrates (1-5). Transmission of B19 infection by derivatives produced from pooled plasma has been reported thus indicating the presence of the infectious virus despite routine viral inactivation/removal procedures during the manufacturing process (6-12). However, the effectiveness against B19 of the current inactivation procedures is unclear due to physical robustness of the nonenveloped virus.

Recently, it has been shown that the genetic diversity of B19 virus is higher than previously expected (13-15; for review 16). Therefore, the species B19 is now subdivided into three different genotypes, with all viruses previously classified as B19 belonging to genotype 1. Until now, genotype 2 DNA has been detected in several European countries including Germany, and the United States (14, 17, 18), whereas detection of B19 genotype 3 was mainly limited to France (15). According to current data, genotype 2 and genotype 3 viral infections cause the same spectrum of illnesses as "classical" B19 infections (15). Given the relatively high homology between the viral proteins, sero-

Correspondence to:
Anna Maria Eis-Hübinger, Prof. Dr.
Institut für Medizinische Mikrobiologie und Immunologie
Universitätsklinikum Bonn
Sigmund-Freud-Str. 25
D-53105 Bonn
Germany
Tel.: +49 228 287 5881, Fax: +49 228 287 4433
E-mail: Anna-Maria.Eis-Huebinger@ukb.uni-bonn.de

Received April 14, 2004
Accepted after revision July 13, 2004

Financial support
This work was carried out with financial support from the Commission of the European Community (Grant QLK2-CT-2001-00877).

Prepublished online September 7, 2004 DOI: 10.1160/TH04-04-0229

logical cross-reaction and a certain degree of cross-protection between the three genotypes could be expected, at least in immunocompetent individuals.

Since there is no information on the frequency of contamination with B19 genotype 2, we investigated, by nested polymerase chain reaction, 202 lots of coagulation factor concentrates representing 21 commercially available products for both genotype 2 and genotype 1 DNA. One hundred and eighty-one lots (13 different products) were currently used concentrates administered during the last three years and 21 lots (8 different products) were formerly used concentrates administered up to the beginning of the 1980s and not virally inactivated. In case of a positive genotype 2 result, DNA sequence analysis was performed. The viral load of genotype 1 DNA in currently and formerly administered concentrates was quantitatively measured by real-time polymerase chain reaction.

Table 1: Detection of parvovirus B19 genotype 1 and genotype 2 DNA in currently administered coagulation factor concentrates.

Coagulation factor	Product	Virus inactivation	IU[1]	No. of lots tested	PCR results: No. of positive lots (%) Genotype 1	Genotype 2
Factor VIII	A	S/D & dry heat 80 °C 72 h	1000	35	7	0
			500	7	4	0
				Σ 42	11	0
	B	Tween 80 & vapour heat 60 °C 10 h	1000	16	12	0
			500	8	4	0
			250	4	0	0
				Σ 28	16	0
	C	S/D & dry heat 100 °C 0.5 h	1000	25	20	2*
			500	2	0	0
				Σ 27	20	2
	D	S/D	1000	11	3	0
	E	Pasteurisation 60 °C 10 h	1000	7	1	0
			500	2	0	0
				Σ 9	1	0
	F	Pasteurisation 60 °C 10 h	1000	10	7	0
	G	S/D & dry heat 80 °C 72 h	1000	1	1	0
	H	S/D & dry heat 100 °C 0.5 h	250	1	1	0
				Total 129	60 (46.5%)	2 (1.6%)
Factor IX	I	Tween 80 & vapour heat 60 °C 10 h, 80 °C 1 h	1000	14	5	0
			600	4	2	0
				Σ 18	7	0
	J	S/D & nanofiltration	1000	10	4	0
			500	3	1	0
				Σ 13	5	0
	K	S/D	500	2	0	0
				Total 33	12 (36.4%)	0
Factor VII	L	Vapour heat 60 °C 10 h, 80 °C 1 h	500	2	0	0
Act. prothrombin complex concentrate	M	Vapour heat 60 °C 10 h, 80 °C 1 h	1000	8	3	0
			500	9	2	0
				Total 17	5	0
All products				Total 181	77 (42.5%)	2 (1.1%)

[1] IU = International Units coagulation factor per vial
* Genotype 2 DNA positive lots referred to as C_1 and C_2

Materials and methods

Coagulation factor concentrates

In total, 202 lots of 21 commercially available plasma-derived coagulation factor concentrates were investigated. One hundred and eighty-one lots (13 different products) were currently available concentrates, administered during the last three years (specimens collected between October 18, 2000 and February 28, 2003), compared to 21 lots (8 different products) taken from formerly used concentrates, administered until the beginning of the eighties and not virally inactivated. Details of the investigated coagulation factor concentrates are given in Table 1 and Table 2.

DNA isolation and polymerase chain reaction

DNA was prepared from 200 µl of reconstituted factor by spin column procedure (QIAamp DNA® Mini Kit, Qiagen, Hilden, Germany) according to the instructions of the manufacturer. Nested polymerase chain reaction (PCR) for detection of B19 genotype 1 DNA was performed as described previously (19). The assay was found to reliably detect the presence of 2 genome equivalents per reaction. PCR was specific for genotype 1 DNA. Amplification of genotype 2 DNA was performed using the primers described by Hokynar et al. (14). PCR was carried out in 50 µl volumes with the following concentration of reagents: 250 µM of each deoxynucleoside triphosphate (Ultrapure dNTPs, Amersham Biosciences, Freiburg, Germany), 25 pmol of each primer (Sigma-Genosys, Steinheim, Germany), 5 µl 10× PCR-buffer (Expand High Fidelity PCR System, Roche Diagnostics, Mannheim, Germany), 2 mM $MgCl_2$ (Roche Diagnostics) and 1.75 U DNA polymerase (Expand High Fidelity PCR System, Roche), and 5 µl of DNA preparation. From first round reaction mixture, 5 µL were transferred to the second round reaction mixture containing the same constituents as the first round mix, except for the nested primers. Amplification was as follows: 95 °C for 5 min followed by 35 cycles each consisting of 94°C for 10 s, 50°C for 10 s, and 72°C for 20 s (T3 Thermocycler, Biometra®, Göttingen, Germany). A final elongation step followed for 3 min at 72°C. Identical conditions were used for the first and second round amplification. The assay accurately detects 2 genome equivalents per sample, determined as mentioned below. Figure 1 illustrates the positions of the amplified regions in the B19 genome.

10 µl of the second-round PCR mixture were analysed by electrophoresis on agarose composite minigels of 1.5%NuSieve® GTG® (FMC)/0.5%SeaKem® LE (FMC) (Cambrex, supplied by Biozym, Hessisch Oldendorf, Germany). Amplified products were visualised by ethidium bromide staining and UV illumination. Positive and negative controls were included in every run. For negative control, all PCR reagents and sterile bidistilled water instead of the sample was used. Strict precautions to avoid contaminations were taken.

DNA sequence analysis

For DNA sequence analysis, half of the genome was amplified by nested PCR using genotype-specific oligonucleotide primers (Fig. 1). For sequencing of genotype 1 DNA, all four nested primer pairs described by Hemauer et al. (20; amplification regions: NS1-C, ΔV, VP1/VP2 and VPC) were used. Additionally, primers for amplification of the genome region between amplification regions VP1/VP2 and VPC (region VPint) were used: outer forward 5′-ACAATGCCAGTGGAAAGGAG-3′ (nucleotide (nt) 3318-3337; all positions according to B19 genotype 1 strain Au, GenBank accession no. M13178) (21); outer reverse 5′-CCCAGGGCGTAAGGA-

Table 2: Detection of parvovirus B19 genotype 1 and genotype 2 DNA in coagulation factor concentrates administered until the beginning of the 1980s.

Coagulation factor	Product	IU[§]	No. of lots tested	PCR results: No. of positive lots (%)	
				Genotype 1	Genotype 2
Factor VIII	a	250-1100	8	6	0
	b	250 / 500	4	4	1*
	c	250 / 500 / 1000	4	2	0
	d	1000	1	1	1
	e	250	1	1	0
	f	1000	1	1	0
	g	500	1	1	1
	h	1000	1	1	0
			Σ 21	17 (81%)	3 (14%)

§ IU = International Units coagulation factor per vial
* Lot contains 500 IU

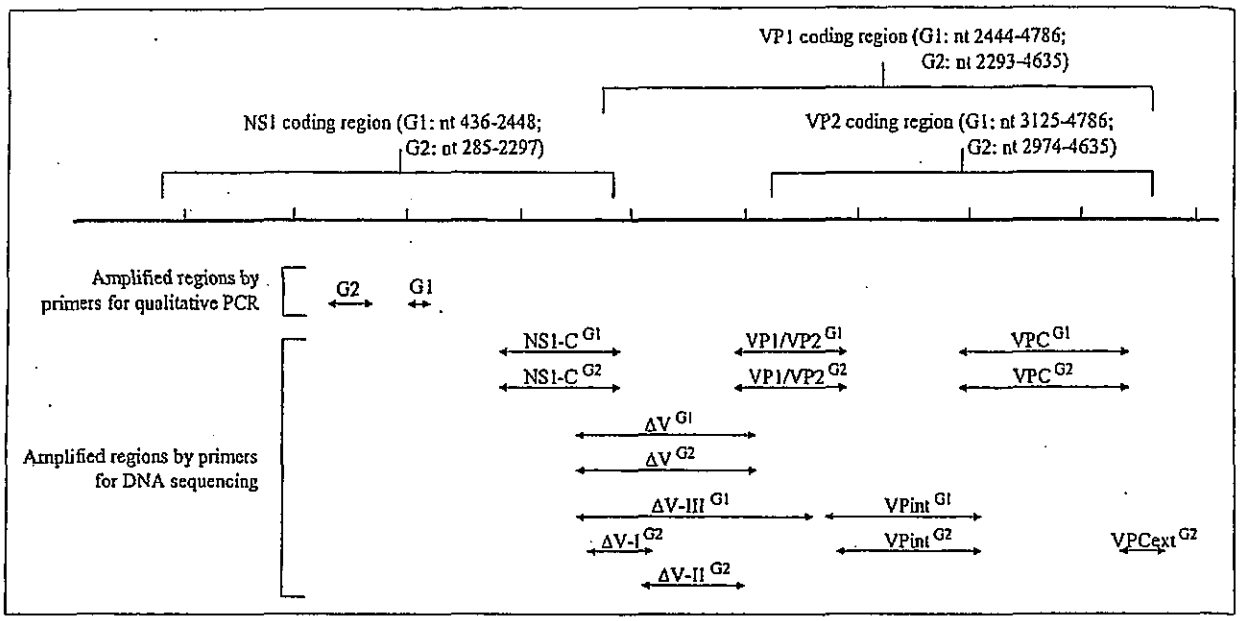


Figure 1: Schematic representation of the amplified regions of the parvovirus B19 genome. The reading frames of the viral proteins NS1, VP1 and VP2 are indicated in the upper part of the figure. Numbering of the nucleotides of B19 genotype 1 (G1) according to the genotype 1 strain Au (GenBank accession no. M13178); numbering of the nucleotides of genotype 2 (G2) according to strain LaLi (GenBank accession no. AY044266). The genome regions amplified by nested PCR using genotype 1 (G1) and genotype 2 (G2) -specific primers are shown in the lower part of the figure. G1- and G2-specific primers used for qualitative PCR were previously described (14, 19); G1- and G2-specific primers for DNA sequencing as mentioned in *material and methods*.

TATT-3′ (nt 4117-4099); nested forward 5′-AAGGTTTGCACCATCAGTCC-3′ (nt 3341-3360); nested reverse 5′-TTAAGGCTTTTCCAGCTCCA-3′ (nt 4064-4045). In cases where no PCR product was obtained by ΔV-specific primers, the following primer set was used (amplification region ΔV-III): outer forward 5′-CTACACACCTTTGGCAGACC-3′ (nt 2151-2170); outer reverse 5′-GGACTGATGGTGCAAACCTT-3′ (nt 3360-3341); nested forward 5′-TTTACCTGTGTGTTGTGTGCAA-3′ (nt 2223-2244); nested reverse 5′-CTGCGGGAGAAAACACCTTA-3′ (nt 3305-3286).

For amplification of genotype 2 DNA the sequences of the primers used for the genotype 1 amplification regions NS1-C, ΔV, VP1/VP2 and VPC were modified according to the sequence of the genotype 2 strain LaLi (14; GenBank accession no. AY044266). Primers for amplification of the genome region between amplification region VP1/VP2 and VPC (amplification region VPint) were as follows: outer forward 5′-CAGTGGAAAAGAGGCAAAGG-3′ (nt 3174-3193; nucleotide positions according to genotype 2 strain LaLi; note that homologous genome regions of genotype 1 and 2 are not congruently numbered); outer reverse 5′-CCAGTGATGGTATGGCTGTG-3′ (nt 3993-3974); nested forward 5′-CATAATGGGCTACTCAACACCA-3′ (nt 3210-3231); nested reverse 5′-GCGCCTGTATTGGAAGTGTC-3′ (nt 3899-3880). When the modified primers failed to amplify the region ΔV the following primer sets were used: amplification region ΔV-I: outer forward 5′-ATTGCCTGTTTGTTGTGTGC-3′ (nt 2072-2091); outer reverse 5′-ATAGGTCTGGAGAGTCTTTAAGATTAC-3′ (nt 2521-2495); nested forward 5′-TGTCCTCATTGTATTAATGTGGGA-3′ (nt 2127-2150); nested reverse 5′-CAAACAGGGAAGATGGGTTT-3′ (nt 2473-2454); amplification region ΔV-II: outer forward 5′-AGGATGTGTATAAGCAATTTGTA-3′ (nt 2342-2364); outer reverse 5′-CTTTTCTGAGGCGTTGTATGC-3′ (nt 2964-2944); nested forward 5′-GTTACTGGGACAGACTTAGAGCTTATA-3′ (nt 2380-2406); nested reverse 5′-ATCTTTTACTGCTTGTGCTTGAA-3′ (nt 2877-2855). For amplification of the region encoding the extreme C-terminal region of the viral structural proteins, the following primers were used (amplification region: VPCext): outer forward 5′-TGGACCAATTGGGGGTATTA-3′ (nt 4315-4334; positions according to genotype 2 strain A6 clone c2, GenBank accession no. AY064475 (17); corresponding sequences of strain LaLi not available); outer reverse 5′-GTTCTCTGCGGGGTATTGG-3′ (nt 4683-4665); nested forward 5′-GAATCCACAGCCTGGAGTGT-3′ (nt 4432-4451); nested reverse 5′-TCTGGGTGGTACAGGAGGAC-3′ (nt 4649-4630).

For DNA sequencing, nested PCR products were purified by QIAquick PCR Purification Kit (Qiagen, Hilden, Germany) and sequencing reactions were carried out using approximately 5-20 ng of the purified PCR product. Amplicons from at least two independent PCR reactions were sequenced in the forward and

reverse directions using the nested primers. Sequencing was performed with the ABI PRISM® BigDye™ Terminator v1.1 Cycle Sequencing Ready Reaction Kit (ABI, Applied Biosystems, Weiterstadt, Germany), unincorporated dye terminators removed using SigmaSpin™ Post-Reaction Clean-Up Columns (Sigma.Aldrich, Steinheim, Germany) and reactions were run on an ABI PRISM® 310 Genetic Analyzer (Applied Biosystems). Sequencing data were manually reviewed. Alignments were generated by ClustalX 1.81 and sequence editing was performed using BioEdit.

Quantitative polymerase chain reaction

For quantitative measurement of genotype 1 DNA contamination, real-time PCR (LightCycler – Parvovirus B19 Quantification Kit, Roche Diagnostics, Mannheim, Germany) was carried out, using a LightCycler instrument (Roche Molecular Biochemicals, Mannheim, Germany), according to the manufacturers instructions. In addition to the Roche B19 DNA standards, the International Standard for B19 DNA (1st World Health Organization International Standard 99/800 for Parvovirus B19 DNA [National Institute of Biological Standard and Control (NIBSC), London, UK]; 5x 10^5 IU per vial) (22) was included in each run. The International Standard was assayed undiluted and in 5 serial tenfold dilutions. The assay amplifies genotype 1 virus. For semi-quantitative measurement of genotype 1 DNA, 10-fold dilutions of the DNA preparations from the factor concentrates, the International Standard for B19 DNA (99/800) and from cloned (almost full-length) B19 DNA were performed. The plasmid pGEM-1/B19 was kindly provided by Dr. Jonathan P. Clewley, Central Public Health Laboratory, London, UK. Nested PCR was performed as mentioned above (19). For semi-quantitative measurement of genotype 2 DNA, a PCR product amplified by the outer primers described by Hokynar et al. (14) was TA cloned into the pCR®4-TOPO® plasmid. Nested PCR with 10-fold dilutions of known amounts of cloned genotype 2 DNA was carried out using the primers described by Hokynar et al. (14) and the amplification protocol given above.

Statistical analysis

The statistical analysis was performed using the χ^2 test.

Results

Contamination with B19 genotype 1 DNA

Genotype 1 DNA was detected by genotype 1-specific PCR in 77/181 (42.5%) lots of coagulation factor concentrates used for therapy in the last three years (Table 1). The percentage of contaminated lots was higher for factor VIII concentrates (47%) than for factor IX (36%) and activated prothrombin complex concentrates (29%). However, the differences were not statistically significant because of the relatively small numbers of factor IX and prothrombin complex concentrate lots. The highest contamination rate was found in product C (74.1%, factor VIII concentrate). Analysis of coagulation factor VIII concentrates used until the beginning of the eighties for therapy showed that the frequency of contamination was significantly higher (17/21 = 81%; $p < 0.01$) than in currently used factor VIII concentrates (Table 2).

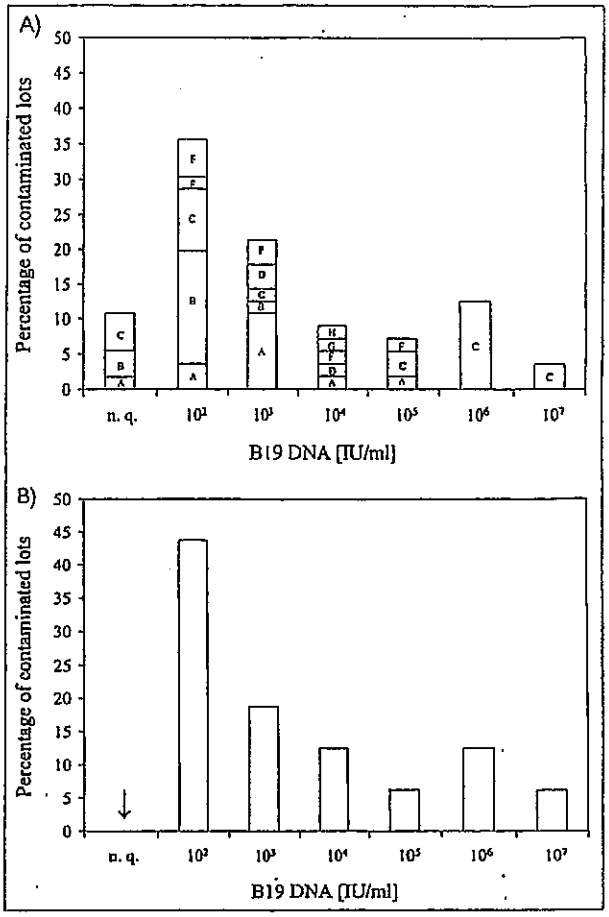


Figure 2: Levels of B19 genotype 1 DNA contamination in coagulation factor VIII concentrates. All PCR-positive lots were quantified by real-time PCR and classified in log10 levels (x-fold value not considered) with the exception of five lots. Figure 2A. Results from 56 currently used lots (four PCR-positive lots not tested). Specific products are indicated by letters in each column. Figure 2B. Results from 16 lots administered until the beginning of the 1980s. Except for one product (product f), all PCR-positive lots were tested. n.q. = not quantifiable.

Quantitative real-time PCR amplifying genotype 1 DNA was performed on 56/60 PCR-positive factor VIII lots currently used and on 16/17 PCR-positive previously used factor VIII lots (Fig. 2). The results showed that contamination ranged from less than 2x 10^2 IU/mL to 3x 10^7 IU/mL. Sixteen percent (9/56) of currently used concentrates proved to be highly contaminat-

Coagulation factor		Genotype 1 (genome equivalents/mL*)	Genotype 2 (genome equivalents/mL)
Currently administered concentrates	C_1	$10^{7\#}$	10^2
	C_2	10^3	10^5
Formerly administered concentrates	b	10^4	10^2
	d	10^5	10^2
	g	10^7	10^5

* 1 genome equivalent is equal to about 0.3 IU of B19
\# x-fold value not considered

Table 3: Semi-quantitative PCR analysis of parvovirus B19 genotype 1 and 2 DNA in co-contaminated clotting factor concentrates.

ed (10^6 to 10^7 IU/ml). The level of B19 DNA contamination was similar in currently and previously used factor concentrates. In factor IX and prothrombin complex concentrate contaminated lots, viral titres were mostly lower (11/12 and 5/5 lots, respectively, analysed). The maximum viral titre observed in factor IX concentrates was 10^5 IU/ml, and 10^3 IU/ml in prothrombin complex concentrates (data not shown).

Contamination with B19 genotype 2 DNA

Genotype 2 DNA was detected by genotype 2-specific PCR in 2/181 (1.1%) lots of currently administered concentrates (Table 1) and in 3/21 (14%) lots of previously used concentrates (Table 2). Thus, the number of concentrates contaminated with genotype 2 DNA (5/202) was significantly lower than the number of concentrates contaminated with genotype 1 DNA (94/202; $p < 0.001$). All genotype 2 DNA positive lots were co-contaminated with genotype 1 DNA.

To verify the PCR results, DNA sequence analysis was carried out on the five double-PCR positive concentrates using genotype 1 and genotype 2-specific primers in separate sequencing reactions. Because of the low degree of genetic variability of parvovirus B19, sequencing of larger parts of the viral genome was performed. Of the ten isolates, seven isolates (4x genotype 1 and 3x genotype 2 isolates) were sequenced over approximately half of the genome (genotype 1: nt 1901-4708; genotype 2: nt 1901-4830; numbering of nt positions according to genotype 1 prototype strain Au, GenBank accession no. M13178). One genotype 2 DNA isolate was sequenced for 2155 nucleotides (nt 2302-2604, 2973-4830; deletion of six consecutive nucleotides coding for 2 amino acids in the nonstructural 11kD protein). However, due to the considerable quantitative differences in viral contamination between genotype 1 and genotype 2 DNA in two products (see below), successful amplification of larger parts of the low-level contaminating genotype virus was not possible. One genotype 1 isolate could only be sequenced over a region of 677 nucleotides (nt 3361-4037) and one genotype 2 contaminant over 1918 nucleotides (nt 1901-2604, 2973-4008, 4653-4830). Nucleotide sequence comparison was performed with genotype 1 strain Au and genotype 2 strain A6 (accession no. AY064475, AY064476).

Sequence analysis revealed that the double-PCR positive concentrates contained both typical genotype 1 and genotype 2 variants. All genotype 1 and genotype 2 DNA isolates differed from each other. Two of the four genotype 1 isolates that were sequenced over half of the genome revealed ambiguities in 7 and 9 nucleotide positions, respectively. These ambiguities were most probably caused by the presence of several B19 strains within the plasma pool.

In genotype 1 isolates, the percentage of nucleotide positions divergent to genotype 1 prototype strain Au varied from 0.46 to 1.35% (the isolate that could not be sequenced for a larger stretch was not considered). Sequence divergence of the three genotype 2 genomes sequenced for half of the genome to the genotype 2 strain A6 was 1.6, 1.7 and 2.3%, respectively. Genetic difference between the three factor-derived genotype 2 genomes and the genome of genotype 1 prototype strain Au was 8.7 (two isolates) and 9.0% and, therefore, within the range of the divergence between genotype 1 strain Au and genotype 2 strain A6 (9.4%). The nucleotide sequence data from this study have been deposited in the nucleotide database of NCBI (National Center for Biotechnology Information) (GenBank accession numbers AY661660-AY661670. Semi-quantitative PCR analysis of the co-contaminated lots revealed relative differences in viral contamination between the two ge-notypes except for one concentrate. In four concentrates genotype 1 DNA was present in higher concentrations than genotype 2 DNA while in one concentrate (C_2) genotype 2 DNA was present in the higher concentration (Table 3). Because there exists no international standard for genotype 2 DNA, the concentration of genotype 1 DNA is also expressed in genome equivalents to provide better comparability.

Discussion

The purpose of the study presented here was to gain insight into the possible risk of contamination of clotting factor concen-

trates by the recently discovered human parvovirus B19 variant, classified as B19 genotype 2. The results of the study clearly show that genotype 2 DNA is present in coagulation factor concentrates much less frequently than genotype 1 DNA. In currently used coagulation factors the detection rate for genotype 2 was 1.1% whereas the rate for genotype 1 was 42.5% ($p < 0.001$). Although the number of investigated products that have been formerly used was rather small, the study further indicates that the rate of genotype 1 DNA contamination in currently used factor VIII products is significantly lower than in previously used products ($p < 0.01$). Moreover, the fact that genotype 2 DNA has been detected in products used up until the early 1980s indicates that the "new" genotype does not represent a recently emerged virus as might have been assumed due to its recent identification (14, 15, 17).

In literature there is only one report describing prevalence of genotype 2 DNA in human blood. Nguyen et al. (17) tested 62 plasma pools each derived from plasma from 2000 Danish voluntary blood donors. No genotype 2 viraemic pool was detected by PCR. In contrast, screening of the plasma pools for B19 identified 40 pools (65%) containing B19 DNA. Furthermore, among 207 serum samples submitted to the NIH specifically for testing for B19 between 1991 and 2001, only one sample collected from an Italian HIV-positive patient with chronic anaemia tested positive for genotype 2 DNA. Thus, the low detection rate of genotype 2 DNA in clotting factor concentrates observed in the present study is consistent with the low frequency of genotype 2 DNA in blood and plasma pools.

In contrast to the low detection rate of genotype 2 DNA in blood, we and others have shown that genotype 2 DNA is present in human tissue in a relatively high proportion. Hokynar et al. (14) detected genotype 2 DNA in 9/19 (47%) human skin samples collected from B19 seropositive individuals. Furthermore, we detected genotype 2 DNA in 27/88 (31%) liver specimens collected from randomly selected adults undergoing liver transplantation or liver biopsy or obtained from autopsied individuals (18). Genotype 2 DNA has also been found in 5/83 (6%) livers from patients with fulminant hepatitis or hepatitis-associated aplastic anaemia (23). These findings indicate that genotype 2 is more widespread than might be suspected from the low detection rate in blood or blood-derived coagulation factor concentrates and that genotype 2, like genotype 1, persists in human tissue (24-26).

With regard to the high incidence of viral DNA in tissue and the low detection rate in blood products one can speculate that the characteristics of the viraemic phase of infection might be different between the two genotypes. B19 infection is characterised by a high-level viraemia (up to 10^{12} genome equivalents/ml) during the early stage of infection which is frequently followed by a low-level viraemia, existing for months or even years after acute disease (27). Genotype 2 viraemia, however, might be shorter and viral titres may be mostly lower than in genotype 1 viraemia resulting in a low occurrence rate in blood products.

Alternatively, it is conceivable that divergence between the structural proteins of the two genotypes mediates an altered sensitivity to the virus removal/inactivation procedures used during the manufacturing of coagulation factor concentrates. However, this hypothesis seems unlikely because the sequence divergence is relatively low, i.e. 1.4 to <2% at the amino acid sequence level for the major viral capsid protein (VP2) which accounts for about 95% of the viral capsid, and 2.2 to 3.3% for the minor viral capsid protein (VP1) present in the virion. This calculation is based on the genotype 2 sequences presented herein, together with those available from GenBank. However, to unambiguously rule out the possibility that the small differences in capsid composition between the two genotypes would mediate a different sensitivity to physical and chemical procedures, specific culture studies with genotype 2 would be necessary. Although there is some experience from work with genotype 1 (28-32), successful propagation of genotype 2 in cell culture as a prerequisite for such investigations has not been reported up to now.

Genotype 1 viral DNA is frequently present in currently administered factor concentrates. The occurrence of blood donations contaminated with genotype 1 DNA has been estimated to be between 1: 5950 to about 1: 30000 (33, 34), increasing to as high as 1: 260 during epidemic periods (35). The high levels of viraemia in acutely infected individuals combined with the resistance of the virus to inactivation procedures, means that there is a high probability of lot contamination. Factor VIII products were found to contain the highest degree of genotype 1 contamination (10^6 and 10^7 IU/ml). However, these maximum levels were present only in the product from one manufacturer (product C). Over the last few years, nucleic acid testing (NAT) of plasma pools for B19 DNA has been increasingly implemented. In 2002 it was stated by a plasma protein consortium (PPTA) that NAT for B19 DNA has now became universally effective and manufacturing pools will not exceed levels of 10^5 IU DNA/ml. Furthermore, although a recommendation was presented suggesting a standardised B19 NAT schedule to ensure that the proposed limit of the FDA for manufacturing pools (<10^4 IU/ml) can be achieved, the application of NAT assays to plasma pools, destined for production of coagulation factor concentrates, remains a voluntary procedure. However, the fact that the overall frequency of viral contamination in currently administered products is significantly lower than in formerly used ones might be interpreted as a positive effect of the present procedures, including NAT, to reduce the risk of contamination of clotting factor products.

The results presented here indicate that genotype 2 is not a frequent contaminant (2.5%) in coagulation factor concentrates. Nevertheless, to further improve the viral safety of blood products it seems reasonable to reflect on the need for implementa-

tion of nucleic acid testing for genotype 2. Since parvovirus B19 has been recognized as a major contaminant of blood products, plasma pool testing by NAT for genotype 1 is now widely applied. However, it can be assumed that, similar to our standard PCR for B19, many primers currently used for B19 PCR do not detect genotype 2 DNA because of insufficient complementarity. Thus, for detection of genotype 2, and possibly genotype 3 DNA, alternative primers such as consensus primers or degenerate primers should be used if separate amplification is to be avoided. Since there is evidence that genotype 2 causes the same spectrum of diseases as genotype 1, development of a PCR system able to detect DNA from both genotypes of the human parvovirus B19 would give an added level of safety to blood products.

Acknowledgement

The authors would like to express their gratitude to Ulrike Reber for excellent technical assistance.

References

1. Zakrzewska K, Azzi A, Patou G, et al. Human parvovirus B19 in clotting factor concentrates: B19 DNA detection by the nested polymerase chain reaction. Br J Haematol 1992; 81: 407-12.
2. Lefrère JJ, Mariotti M, Thauvin M. B19 parvovirus DNA in solvent/detergent-treated anti-haemophilia concentrates. Lancet 1994; 343: 211-2.
3. Saldanha J, Minor P. Detection of human parvovirus B19 DNA in plasma pools and blood products derived from these pools: implications for efficiency and consistency of removal of B19 DNA during manufacture. Br J Haematol 1996; 93: 714-9.
4. Eis-Hübinger AM, Sasowski U, Brackmann HH. Parvovirus B19 DNA contamination in coagulation factor VIII products. Thromb Haemost 1999; 81: 476-7.
5. Schmidt I, Blümel J, Seitz H, et al. Parvovirus B19 DNA in plasma pools and plasma derivatives. Vox Sang 2001; 81: 228-35.
6. Bartolomei Corsi O, Azzi A, Morfini M, et al. Human parvovirus infection in haemophiliacs first infused with treated clotting factor concentrates. J Med Virol 1988; 25: 165-70.
7. Lyon DJ, Chapman CS, Martin C, et al. Symptomatic parvovirus B19 infection and heat-treated factor IX concentrate. Lancet 1989; 1: 1085.
8. Yee TT, Cohen BJ, Pasi KJ, et al. Transmission of symptomatic parvovirus B19 infection by clotting factor concentrate. Br J Haematol 1996; 93: 457-9.
9. Santagostino E, Mannucci PM, Gringeri A, et al. Transmission of parvovirus B19 by coagulation factor concentrates exposed to 100°C heat after lyophilization. Transfusion 1997; 37: 517-22.
10. Azzi A, Morfini M, Mannucci PM. The transfusion-associated transmission of parvovirus B19. Transfus Med Rev 1999; 13: 194-204.
11. Hino M, Ishiko O, Honda KI, et al. Transmission of symptomatic parvovirus B19 infection by fibrin sealant used during surgery. Br J Haematol 2000; 108 194-5.
12. Blümel J, Schmidt I, Effenberger W, et al. Parvovirus B19 transmission by heat-treated clotting factor concentrates. Transfusion 2002; 42: 1473-81.
13. Nguyen QT, Sifer C, Schneider V, et al. Detection of an erythrovirus sequence distinct from B19 in a child with acute anaemia. Lancet 1998; 352: 1524.
14. Hokynar K, Söderlund-Venermo M, Pesonen M, et al. A new parvovirus genotype persistent in human skin. Virology 2002; 302: 224-8.
15. Servant A, Laperche S, Lallemand F, et al. Genetic diversity within human erythroviruses: identification of three genotypes. J Virol 2002; 76: 9124-34.
16. Gallinella G, Venturoli S, Manaresi E, et al. B19 virus genome diversity: epidemiological and clinical correlations. J Clin Virol 2003; 28: 1-13.
17. Nguyen QT, Wong S, Heegaard ED, et al. Identification and characterization of a second novel human erythrovirus variant, A6. Virology 2002; 301: 374-80.
18. Schneider B, Reber U, Tolba R, et al. Prevalence of human parvovirus genotype 1 and genotype 2 DNA in livers and bone marrows of adults. Annual Meeting of GfV – Joint Meeting with SIV, March 17-20, Tübingen, Germany.
19. Eis-Hübinger AM, Sasowski U, Brackmann HH, et al. Parvovirus B19 DNA is frequently present in recombinant coagulation factor VIII products. Thromb Haemost 1996; 76: 1120.
20. Hemauer A, von Poblotzki A, Gigler A, et al. Sequence variability among different parvovirus B19 isolates. J Gen Virol 1996; 77: 1781-5.
21. Shade RO, Blundell MC, Cotmore SF, et al. Nucleotide sequence and genome organization of human parvovirus B19 isolated from the serum of a child during aplastic crisis. J Virol 1986; 58: 921-36.
22. Saldanha J, Lelie N, Yu MW, et al. Establishment of the first World Health Organization International Standard for human parvovirus B19 DNA nucleic acid amplification techniques. Vox Sang 2002; 82: 24-31.
23. Wong S, Young NS, Brown KE. Prevalence of parvovirus B19 in liver tissue: no association with fulminant hepatitis or hepatitis-associated aplastic anemia. J Infect Dis 2003; 187: 1581-6.
24. Söderlund M, von Essen R, Haapasaari J, et al. Persistence of parvovirus B19 DNA in synovial membranes of young patients with and without chronic arthropathy. Lancet 1997; 349: 1063-5.
25. Cassinotti P, Burtonboy G, Fopp M, et al. Evidence for persistence of human parvovirus B19 DNA in bone marrow. J Med Virol 1997; 53: 229-32.
26. Eis-Hübinger AM, Reber U, Abdul-Nour T, et al. Evidence for persistence of parvovirus B19 DNA in livers of adults. J Med Virol 2001; 65: 395-401.
27. Musiani M, Zerbini M, Gentilomi G, et al. Parvovirus B19 clearance from peripheral blood after acute infection. J Infect Dis 1995; 172: 1360-3.
28. Ozawa K, Kurtzman G, Young N. Replication of the B19 parvovirus in human bone marrow cell cultures. Science 1986; 233: 883-6.
29. Srivastava A, Lu L. Replication of B19 parvovirus in highly enriched hematopoietic progenitor cells from normal human bone marrow. J Virol 1988; 62: 3059-63.
30. Munshi NC, Zhou S, Woody MJ, et al. Successful replication of parvovirus B19 in the human megakaryocytic leukemia cell line MB-02. J Virol 1993; 67: 562-6.
31. Blümel J, Schmidt I, Willkommen H, et al. Inactivation of parvovirus B19 during pasteurization of human serum albumin. Transfusion 2002; 42: 1011-8.
32. Yunoki M, Tsujikawa M, Urayama T, et al. Heat sensitivity of human parvovirus B19. Vox Sang 2003; 84: 164-9.
33. Aubin JT, Defer C, Vidaud M, et al. Large-scale screening for human parvovirus B19 DNA by PCR: application to the quality control of plasma for fractionation. Vox Sang 2000; 78: 7-12.
34. Cohen BJ, Field AM, Gudnadottir S, et al. Blood donor screening for parvovirus B19. J Virol Methods 1990; 30: 233-8.
35. Brown KE, Young NS, Alving BM, et al. Parvovirus B19: implications for transfusion medicine. Summary of a workshop. Transfusion 2001; 41: 130-5.

医薬品　研究報告 調査報告書

識別番号・報告回数			報告日	第一報入手日	新医薬品等の区分	総合機構処理欄
一般的名称	－		研究報告の公表状況	AABB Weekly Report2006:12(32)10.	公表国 米国	
販売名(企業名)	－					

研究報告の概要	使用上の注意記載状況・その他参考事項等
自動車事故後に輸血を受けた患者 1 例がその後マラリアと診断され、疑わしい血液を認識していたにもかかわらず、流通を防げなかったとして韓国赤十字は批判を受けている。 不適切な血液スクリーニング手順にも関わらず韓国赤十字は、プライバシーに関する法律をもとにマラリア感染率が高い地域に関する情報を韓国 CDC (Korea Center for Disease Control and Prevention) が提供しなかったことが原因であるとしている。	

報告企業の意見	今後の対応
韓国において、輸血によりマラリアが感染したとの報告である。 血漿分画製剤によりマラリアが感染したとの報告はない。 なお、日本赤十字社では、輸血によるマラリア感染を防ぐため、WHO の指定しているマラリア流行地域に旅行した人については 1 年間、居住した人については 3 年間、献血を禁止している。	今後ともマラリアに関する安全性情報等に留意していく。

An article in the *Japan Times* further noted that the government was found liable for failing to prevent the use of these products. In handing down his ruling, Judge Keiji Suda said that the state and the company were negligent because they allowed the use of unheated blood products despite the knowledge of the dangers associated with their use.

The recent distribution of blood containing malaria has prompted public concern regarding the Korean National Red Cross and its management of blood and blood products. According to a Sept. 8 *Korea Times* article, a patient who received a transfusion after a car accident and was subsequently diagnosed with malaria has criticized the organization for failing to prevent the distribution of suspect blood. Some allege that the Korean National Red Cross was made aware of the situation but did not take any action, resulting in continued circulation of unsuitable blood products. One patient has reportedly already died after contracting malaria via transfusion.

According to the article, despite inadequate blood screening procedures, the Korean National Red Cross attributes the problem to a privacy law that prevents it from viewing data from the Korea Center for Disease Control and Prevention, which holds information on regions with a high rate of malaria infection.

Industry

Abbott Laboratories will soon deliver 20 of its PRISM system fully automated blood screening instruments to ARC U.S. National Testing Laboratories. In addition to the PRISM systems, the company will also provide the ARC laboratories with the hepatitis B core antibody and surface antigen assays. According to a Sept. 6 press release posted on PR Newswire, the contract for the equipment and assays begins immediately and is expected to run through 2011. "This agreement underscores our longstanding commitment to working with the American Red Cross to help ensure the safety of the nation's blood supply," said Jeff Binder, senior vice president of diagnostic operations at Abbott.

People

Kathy Connolly, chair of the AABB Donor Recruitment and Public Relations Committee, was recently recognized for her commitment to the blood community. Connolly, who began her career in blood banking more than 30 years ago, works as the director of public relations for the Rhode Island Blood Center and was presented with the Bank of America's 2006 Neighborhood Champions in acknowledgment of her dedication to health care. In 2003, Connolly was awarded the AABB Chapman-Franzmeier Memorial Award for her local and national efforts to recruit blood donors.

別紙様式第2-1

No. 3

医薬品 研究報告 調査報告書

識別番号・報告回数			報告日	第一報入手日	新医薬品等の区分	機構処理欄
				2006. 6. 26	該当なし	

一般的名称	(製造承認書に記載なし)	研究報告の公表状況	CDC. 2006 Jun 16; Available from: URL: http://www.cdc.gov/travel/other/2006/malaria_bahamas.htm	公表国 米国
販売名(企業名)	合成血「日赤」(日本赤十字社) 照射合成血「日赤」(日本赤十字社)			

研究報告の概要

○マラリア発生警告(バハマ、グレートエグズーマ島)
2006年6月中旬、米国疾病対策予防センター(CDC)は、バハマのグレートエグズーマ島におけるマラリア発生確認の報告を受領した。当該地域では、通常はマラリアの伝播は起こらず、これまで抗マラリア薬の使用は勧告されていなかった。
6月29日時点で合計18例が発生した。このうち4例は旅行者で、出身国は米国2例、カナダ1例、及びドイツ1例であり、滞在期間は4月後半から5月末の間であった。
これらの症例は、いずれもPlasmodium falciparum による感染症(熱帯熱マラリア)であることが確認されている。感染したバハマ住民の一部は、流行地域であるハイチを最近旅行した可能性がある。6月19日以降は新たな感染例は確認されていない。
CDCは米国からグレートエグズーマ島への旅行者に対して、十分な予防効果のある高用量のクロロキンの使用を勧告している。この勧告は一時的なものであり、バハマ諸島の他の島は該当しない。
抗マラリア薬の予防効果は100%ではないため、当地への旅行者は蚊に刺されないよう予防策を講ずること。当地では、蚊媒介性の他の疾患が起こっているため、バハマ諸島の他の島に向かう旅行者も同様に予防策を取ること。
外出時、特に日没から日出の間には、露出した皮膚の表面に防虫剤を塗布すること。30%～50% DEET(N, N-diethyl-m-toluamide)を含有する防虫剤が推奨される。低濃度の場合は防虫効果が短時間となるため、より頻繁に使用する必要がある。
熱帯熱マラリアは迅速に治療しないと重症となり生命を脅かす疾患である。当地に旅行して、発熱やインフルエンザ様症状を発症した場合、直ちに専門医の治療を受けること。このとき、医療機関でマラリアの発生地域へ旅行した旨を伝えること。

報告企業の意見	今後の対応
米国疾病対策予防センターは、バハマのグレートエグズーマ島におけるマラリア発生を確認し、旅行者に対して抗マラリア薬の使用を勧告したとの報告である。	日本赤十字社は、8月1日以降、バハマに滞在した場合は帰国(入国)から1年間献血延期としている(帰国(入国)後にマラリアを思わせる症状があった場合は、マラリア感染が否定されるまで)。また、今後も引き続き、マラリア感染に関する新たな知見及び情報の収集に努める。

使用上の注意記載状況・その他参考事項等

合成血「日赤」
照射合成血「日赤」

血液を介するウイルス、
細菌、原虫等の感染
vCJD等の伝播のリスク

Outbreak Notice

Update: Malaria, Great Exuma, Bahamas

This information is current as of today, July 4, 2006, 04:39:43 PM

Updated: June 30, 2006
Released: June 16, 2006

In mid-June 2006, the Centers for Disease Control and Prevention (CDC) received official reports of confirmed malaria cases in Great Exuma, Bahamas, an area where malaria transmission does not normally occur and for which antimalarial drugs have not previously been recommended. As of June 29, there have been a total of 18 cases of which 4 were travelers. Of the 4 travel-associated cases, 2 were from the U.S., 1 from Canada, and 1 from Germany. All had traveled to Great Exuma for varying periods between late April and the end of May.

All these confirmed infections were caused by *Plasmodium falciparum*. Most of the patients reported no recent travel to malaria-endemic areas, but some of the Bahamas residents diagnosed with malaria may have recently traveled from Haiti, where *P. falciparum* is endemic. No additional cases of malaria have been identified since June 19.

Malaria is not considered endemic on the islands of the Bahamas. The Ministry of Health in the Bahamas has responded with heightened surveillance for and treatment of malaria cases, mosquito control measures, and education of the local population. The Caribbean Epidemiology Center and the Pan American Health Organization/World Health Organization are assisting the Ministry of Health with these response measures.

Antimalarial Medication

At this time, CDC is recommending that U.S. based travelers take preventive doses of chloroquine before, during, and after they travel to Great Exuma. This recommendation is expected to be temporary and does not apply to other islands of the Bahamas. Chloroquine has a long history of use and safety and is well tolerated by most people, including children. People with an allergy to chloroquine should discuss an alternative antimalarial drug with their health-care provider. To learn more about chloroquine, including dosing information, see Information for the Public: Prescription Drugs for Malaria.

Other Prevention Measures

Because chloroquine and other antimalarial drugs are not 100% protective, travelers to Great Exuma should take precautions to protect against mosquito bites. These prevention measures should be taken by travelers to other islands in the Bahamas as well because other mosquito transmitted infections occur there.

- Use insect repellent on exposed skin surfaces when outdoors, particularly from dusk to dawn. Repellents containing 30% – 50% DEET (N, N-diethyl-m-toluamide) are recommended. Lower concentrations of DEET offer shorter-term protection, requiring more frequent reapplication.
- To learn more about preventing mosquito bites and the appropriate use of insect repellents, visit Protection Against Mosquito and Other Arthropods in *Health Information for International Travel* and What You Need to Know about Mosquito Repellent.

Malaria caused by *P. falciparum* may rapidly result in a severe, life-threatening illness if not promptly treated. If you have traveled to Great Exuma and you become ill with fever and other flu-like

symptoms, you should immediately seek professional medical care; inform your health-care provider that you have visited an area currently experiencing a malaria outbreak.

Visit the CDC's Travelers' Health website for Health information for Travelers to Countries in the Caribbean.

Additional information about malaria can be found at the CDC Malaria homepage.

Health-care providers needing assistance with diagnosis or management of suspected cases of malaria should call the CDC Malaria Hotline: 770-488-7788 (M-F, 8 am-4:30 pm, Eastern Time). For consultation after hours, call 770-488-7100 and ask to speak with a CDC Malaria Branch clinician.

Date: June 30, 2006
Content Source: National Center for Infectious Diseases, Division of Global Migration and Quarantine

SAFER·HEALTHIER·PEOPLE™

Centers for Disease Control and Prevention,1600 Clifton Rd, Atlanta, GA 30333, U.S.A
Tel: (404) 639-3311 / Public Inquiries: (404) 639-3534 / (800) 311-3435

医薬品 研究報告 調査報告書

識別番号・報告回数			報告日	第一報入手日	新医薬品等の区分	機構処理欄
				2006. 7. 3	該当なし	

一般的名称	(製造承認書に記載なし)	研究報告の公表状況		公表国
販売名(企業名)	合成血「日赤」(日本赤十字社) 照射合成血「日赤」(日本赤十字社)		ProMED. 20060624-1758, 2006 Jun 24. 情報源:Kyrgyzstan Press, 2006 Jun 22.	キルギスタン

研究報告の概要

○マラリアーキルギスタン
キルギスタンの首都で79例のマラリア症例が登録された。60例はBishkek地方、19例はChuysk地方の症例だった。予防手段を講じているにもかかわらず、流行は拡大中である。以下の情報はBishkekにある国立衛生疫学監視センター副所長のAdilbek Djuzenovから提供された。マラリア撲滅のための予防活動には数年必要である。キルギスタンでは2005年に125例の症例が登録された。このうちほとんどはBishkek地方のAk-Bata村とKalis ordo村の住民だった。感染の中心は、Chuysk地方のAla-Archinsk貯水池である。Ak-Bata村とKalis ordo村の819世帯で殺虫剤を散布した。しかし、住民に問題を理解させるのは困難で、殺虫剤散布も時々散布が許されるだけである。マラリア治療にはPrimosinが有効で、人道機関から十分な量の薬が送られている。
キルギスタンにおけるマラリアは、1959年に一度撲滅されたものの、1986年以降は、アフガニスタンからの帰還兵が入国するのに伴って毎年国内で感染した症例が登録されている。

報告企業の意見	今後の対応
キルギスタンの首都で79例のマラリア症例が登録され、流行が拡大中であるとの報告である。	日本赤十字社では、輸血感染症対策として問診時に海外渡航歴の有無を確認し、帰国後4週間は献血不適としている。今後も引き続き、マラリア感染に関する新たな知見及び情報の収集、対応に努める。

使用上の注意記載状況・その他参考事項等

合成血「日赤」
照射合成血「日赤」

血液を介するウイルス、細菌、原虫等の感染
vCJD等の伝播のリスク

Back

Archive Number 20060624.1758
Published Date 24-JUN-2006
Subject PRO/EDR> Malaria - Kyrgyzstan (Bishkek)

MALARIA - KYRGYZSTAN (BISHKEK)
****
A ProMED-mail post
<http://www.promedmail.org>
ProMED-mail is a program of the
International Society for Infectious Diseases
<http://www.isid.org>

Date: Sat, 24 Jun 2006
From: ProMED-mail Russian correspondent AP <promed@promedmail.org>
Source: Kyrgyzstan Press [22 Jun 2006; trans. Mod.NR; edited]
<http://pr.kg/n/detail.php?id=3D8576>

In the capital of Kyrgyzstan 79 cases of malaria have been registered; 60 from Bishkek and 19 from the Chuysk region. Despite preventive measures, the outbreak is growing. This information was provided by the deputy chief of the state sanitary epidemiological surveillance (SES) center in Bishkek, Adilbek Djuzenov.

According to Adilbek Djuzenov, it requires several years of preventive work to destroy a malaria focus. Kyrgyzstan registered 125 malaria cases in 2005, of whom most were inhabitants of Ak-Bata and Kalis Ordo villages of Bishkek. The epicenter of the spread is the Ala-Archinsk reservoir in the Chuysk region.

According to Djuzenov, 819 households at Ak-Bata and Kalis ordo have been sprayed with insecticides. However, it is challenging for the population to understand the problem, and [only] sometimes do people allow the SES staff to spray their houses.

According to Adilbek Djuzenov, the disease can be treated with Primosin [ProMED does not know what drug is being referred to under this brand name], and enough drugs were delivered by humanitarian aid.

--
ProMED-mail
<promed@promedmail.org>

[ProMED reported 88 cases of malaria in Bishkek in 2005. We still do not have a species identification. Malaria was eradicated in Kyrgyzstan in 1959; however, from 1986 onwards, as a result of the importation of malaria by military personnel returning from Afghanistan, a few local cases have been registered annually. In 1986 and 1987, 14 and 10 autochthonous malaria cases were detected, respectively. In 1988, 21 cases due to local transmission were registered. In 2002, a total of 2267 autochthonous _P. vivax_ cases were reported in the southwestern regions of the country, including Batken, Osh and Jalal-Abad.

The explosive resumption of malaria transmission in Kyrgyzstan was the result of immigration of a number of infected people from Tajikistan into the Batken region. In 2004-2005, there was a significant decrease in the reported number of autochthonous malaria cases (42 in 2005). However, in 2004 the first autochthonous case of _P. falciparum_ malaria was reported in the Aravan district of the southern part of Kyrgyzstan, in an area bordering Uzbekistan, and in 2005 the number of autochthonous cases of _P. vivax_ malaria increased in the outskirts of Bishkek, the capital of the country (Source: <http://www.euro.who.int/malaria/ctryinfo/affected/20020712_17>).
ProMED reported 88 cases of malaria from Kyrgyzstan in 2005 in

contrast to the 42 the country reported theough WHO. This indicates that there may be some underreporting of cases. - Mod.EP]

[Bishkek is the capital of Kyrgyzstan and its population is 768 000. The Chuysk region borders Bishkek and has 765 700 people. - Mod.NR]

[A good map of Kyrgystan can be found at:
<http://www.lib.utexas.edu/maps/commonwealth/kyrgyzstan_pol96.jpg>. - Mod.MPP]

[see also:
2005

Malaria - Kyrgyzstan (02) 20050823.2489
Malaria - Kyrgyzstan 20050723.2124
2004

Malaria, falciparum - Kyrgyzstan (Osh) 20040815.2263
2002

Malaria, autochthonous - Russia (Moscow) 20020608.4439
2001

Malaria, autochthonous - NIS: 1992-2000 20010819.1962
2000

Malaria, autochthonous - Kazakhstan (02) 20000904.1507
Malaria, autochthonous - Russia (Krasnoyarsk) 20000816.1366
Malaria - Russia (Moscow) 20000525.0827
1999

Malaria, autochthonous - Russia (Ryazan) 19990909.1593
1998

Malaria - Turkmenistan (02) 19981218.2398
Malaria - Kyrgyzstan (02) 19980910.1825
Malaria - Azerbaijan & Newly Independent States 19980714.1332
Malaria, epidemic - Azerbaijan (02) 19980708.1278
Malaria, epidemic - Azerbaijan: RFI 19980706.1269]
.....nr/mpp/ep/pg/mpp

別紙様式第2-1

No. 7

医薬品　研究報告　調査報告書

識別番号・報告回数			報告日	第一報入手日	新医薬品等の区分	機構処理欄
				2006. 5. 24	該当なし	

一般的名称	（製造承認書に記載なし）	研究報告の公表状況	AABB Association Bulletin. 2006 Apr 26.	公表国 米国
販売名（企業名）	合成血「日赤」（日本赤十字社） 照射合成血「日赤」（日本赤十字社）			

研究報告の概要

○AABBがムンプスウイルスの流行に対応して採血施設に勧告
米国中西部でのムンプスの流行に対応して、AABBは感染者と感染者に接触した人を一時的に供血延期とすることを勧告した。輸血によるムンプスウイルスの感染はこれまで確認されていないが、ウイルス血症が起こることは知られている。このため、輸血による感染の可能性を考慮して、AABBの感染症委員会とFDAはムンプス流行地域の採血施設が予防策をとることに合意した。
採血施設への勧告の内容は以下の通りである。
・教育施設での移動採血を実施するかどうか、地元自治体や州の公衆衛生当局に照会すること。
・供血が見込まれる人に対して、疾患についての情報提供を行うこと。
・最近ムンプスに罹患した人については、すべての症状が消えてから14日間供血延期とすること。感染者と接触した人については、覚えている最後の接触から28日間延期すること。
・供血後にムンプスと診断された場合は、症状が消える前28日間および消えた後14日間の供血由来の製品は回収、隔離保管、廃棄を行うこと。供血後に感染者との接触が報告された場合は、覚えている最後の接触後28日間の供血由来の製品は回収、隔離保管、廃棄を行うこと。
・採血施設は、ムンプスが流行している施設あるいは地域での供血由来の新鮮凍結血漿については製造および使用を避け、他の製剤に転用してもよい。
受血者への通知については勧告していないが、輸血によるムンプスウイルス感染のリスクを推定するために行っても構わない。

報告企業の意見	今後の対応
米国中西部でのムンプスの流行に対応して、AABBは感染者と感染者に接触した人を一時的に供血延期とすることを勧告したとの報告である。	日本赤十字社は、問診でムンプスの既往があった場合、治癒後3週間献血不可としている。献血後に感染が判明した場合は、今後も引き続き情報の収集に努める。

使用上の注意記載状況・その他参考事項等

合成血「日赤」
照射合成血「日赤」

血液を介するウイルス、細菌、原虫等の感染
vCJD等の伝播のリスク

Advancing Transfusion and Cellular Therapies Worldwide

ASSOCIATION BULLETIN

#06-04

Date: April 26, 2006

To: AABB Members

From: Christopher D. Hillyer, MD – President
Karen Shoos Lipton, JD – Chief Executive Officer

Re: Recommendations for Blood Collection Facilities in Response to Epidemic Mumps in the Midwest

Background

The state of Iowa is experiencing a large outbreak of mumps that began in December 2005. As of April 20, 2006 more than 1,000 suspect, probable and confirmed cases have been reported to the Iowa Department of Public Health. The majority of infections are among persons 18-25 years of age. Cases initially predominated in postsecondary educational facilities (colleges, universities, trade schools, etc). However, in recent weeks many cases have been reported outside these venues. Additional cases of mumps, possibly linked to the Iowa outbreak, are also under investigation in eight neighboring states, including Illinois, Indiana, Kansas, Michigan, Minnesota, Missouri, Nebraska and Wisconsin. The outbreak is expected to spread further, perhaps nationally.

The source of the current US outbreak is unknown. However, the mumps strain has been identified as serogroup G, the same as that circulating in the United Kingdom (UK). The outbreak in the UK has been ongoing from 2004 to 2006 and has involved more than 70,000 cases. The individuals had been fully immunized [with two doses of Measles-Mumps-Rubella (MMR) vaccine] in approximately 70% of the cases investigated to date.

Mumps is an acute viral infection characterized by a nonspecific prodrome including myalgia, anorexia, malaise, headache and fever, followed by acute onset of unilateral or bilateral tender swelling of parotid or other salivary glands. In unvaccinated populations, an estimated 30-70% of mumps infections are associated with typical acute parotitis. However, as many as 20% of infections are asymptomatic and nearly 50% are associated with nonspecific or primarily respiratory symptoms (cough, sore throat), with or without parotitis.

Complications of mumps infection can include deafness, orchitis, oophoritis, or mastitis (inflammation of the testicles, ovaries or breasts, respectively), pancreatitis,

meningitis/encephalitis and spontaneous abortion. With the exception of deafness, these complications are more common among adults than children.

Natural transmission of mumps virus occurs by direct contact with respiratory droplets (ie, coughing and sneezing), saliva or contact with contaminated fomites. The incubation period is generally 16-18 days (range 12-25 days) from exposure to onset of symptoms. Mumps can be spread from an infected person by droplets for about five days after onset of symptoms; however, virus has been found in the saliva of patients for as long as nine days after onset.

Members of the AABB Transfusion-Transmitted Diseases (TTD) Committee and representatives of the US Food and Drug Administration (FDA) have discussed the potential transfusion transmission of mumps to transfusion recipients from donors with unrecognized infection and asymptomatic viremia. Transfusion transmission of mumps virus has never been observed; however, viremia is known to occur, although the kinetics are poorly characterized. Information suggests that the viremia is in the form of both cell-associated and free virus. Whether transfusion-acquired disease would present with clinical signs that would allow recognition of mumps after transmission by this unnatural route is unknown.

Recommendations

On the basis of the current state of knowledge about the possibility of transfusion-transmitted mumps, the TTD Committee and the FDA agree that a precautionary approach should be adopted by blood collection facilities in areas affected by epidemic mumps until more information is available.

1. Blood drives at postsecondary educational facilities or other similar facilities in areas experiencing epidemic mumps should be scheduled or canceled at the discretion of the collection facility's medical director in consultation with local and/or state public health authorities. In making the decision, medical directors should use information about mumps activity in the general area and at the specific institution where the blood drive is scheduled. Decisions should be consistent with efforts to minimize the risk of the theoretical transmission of mumps yet maintain local and regional blood supplies adequate for medical need.
2. Donor information: Prospective donors in areas experiencing epidemic mumps should be provided with information about the existence of mumps in the local area, the concern about its theoretical transmission by blood, and the donor deferral criteria specified below. This information can be in any of the following forms:
    a. Information provided by recruiters before presentation to donate.
    b. Written information provided at registration that allows self-deferral before screening and/or during administration of the donor history questionnaire.
    c. New questions added to the donor history questionnaire to allow deferral at the time of screening.

These options are similar to those accepted by FDA at the time of the severe acute respiratory syndrome (SARS) epidemic. The decision to implement these measures in all

or part of a blood region should be made by the collection facility medical director in consultation with local and/or state public health authorities and using information about mumps activity in the area.

3. Donor eligibility: A donor is required to be well on the day of donation.
4. Temporary deferral criteria.
    a. Donors with recent mumps infection should be deferred until 14 days after the resolution of all symptoms of infection.
    b. Donors who have had contact with a person or persons with mumps should be deferred until 28 days after the last recognized contact. Contact is defined as any of the following situations:
        i. Living in the same dwelling (eg, house, apartment, dormitory room) as a case patient with a diagnosis of mumps.
        ii. Recognized direct contact with upper respiratory secretions (eg, kissing) or sharing utensils that might be contaminated with upper respiratory secretions (eg, eating utensils, cups, drinking glasses) with a case patient with a diagnosis of mumps.
        iii. Recognized contact within three feet of a case patient with a diagnosis of mumps without the use of barrier precautions (mask and eye protection).
    c. Per the AABB *Standards for Blood Banks and Transfusion Services, 23rd Edition,* receipt of MMR vaccination requires deferral for 28 days.
5. Post donation information: It is probable that if the epidemic is sustained, blood collection facilities will begin to receive post donation information about donors who have developed mumps or who have had recognized contact with mumps that had not been recognized and reported at the time of a prior donation. Because of the relatively long incubation period of mumps, there is a possibility that some of these donors may have been viremic at the time of their donation.
    a. When a donor provides post donation information that he or she has been diagnosed with mumps, the donor should be deferred for 14 days after resolution of all symptoms of infection. Any products collected in the 28 days before or the 14 days after resolution of symptoms should be recalled, quarantined and destroyed unless used for research.
    b. When a donor provides post donation information that he or she was the contact of a mumps case patient, as defined above, the donor should be deferred for 28 days after the last recognized contact. Any products collected from the first date of such contact until 28 days after the last recognized contact should be recalled, quarantined and destroyed, unless used for research.
6. Plasma for further manufacture (source and recovered) is not affected by these recommendations because virus inactivation procedures used to manufacture plasma derivatives should robustly inactivate this enveloped virus.

7. Collection facilities may want to consider refraining from the production and transfusion of fresh frozen plasma from collections from institutions or locales with epidemic mumps and diverting of such plasma for further manufacture. (Relabeling of previously manufactured fresh frozen plasma from such institutions or locales for further manufacture will require a variance from FDA as was required for West Nile virus in 2003.)
8. No recommendation is being made to perform consignee notification for the purpose of recipient notification at this time. However, some blood collection facilities, transfusion services and providers may wish to do so, to facilitate an estimate of the risk, if any, of transfusion transmission of the mumps virus. Appropriate samples for study might include plasma, serum and cells from the index donation for serology, viral culture and nucleic acid amplification, and the same on samples of recipients. Serial samples on the donor and recipients may be useful to study seroconversion and viral kinetics if infection is suspected.
9. Collection facilities that implement measures to prevent the theoretical transmission of mumps virus by transfusion should include notification to the FDA in their annual report.

America's Blood Centers (ABC) and American Red Cross (ARC) concur with these recommendations.

別紙様式第2-1

No. 22

医薬品 研究報告 調査報告書

識別番号・報告回数			報告日	第一報入手日	新医薬品等の区分	機構処理欄
				2006. 6. 20	該当なし	

一般的名称	(製造承認書に記載なし)	研究報告の公表状況	Fischer SA, Graham MB, Kuehnert MJ, Kotton CN, Srinivasan A, Marty FM, Comer JA, Guarner J, Paddock CD, DeMeo DL, Shieh WJ, Erickson BR, Bandy U, DeMaria A Jr, Davis JP, Delmonico FL, Pavlin B, Likos A, Vincent MJ, Sealy TK, Goldsmith CS, Jernigan DB, Rollin PE, Packard MM, Patel M, Rowland C, Helfand RF, Nichol ST, Fishman JA, Ksiazek T, Zaki SR; LCMV in Transplant Recipients Investigation Team. N Engl J Med. 2006 May 25;354(21):2235-49.	公表国
販売名(企業名)	合成血「日赤」(日本赤十字社) 照射合成血「日赤」(日本赤十字社)			米国

研究報告の概要

○臓器移植によるリンパ性脈絡髄膜炎ウイルス(LCMV)の伝播

背景:2003年12月及び2005年4月に固形臓器の移植を受けた2つの患者群を調査したところ、感染症を示唆する兆候と症状が発現した。診断によって得るものがなく、それぞれ共通のドナーが判明したため、各群に対する調査を実施した。

方法: 2名のドナー及び8名の被移植者から採取した試料を、ウイルス培養、電子顕微鏡、血清学的検査、分子解析、及び免疫組織化学染色により調べ、原因を明らかにした。臨床的経過の特徴を調査し、疾患の原因を特定するため、聞き取り調査、環境評価、カルテのレビューを含む疫学調査を実施した。

結果:検査により全ての被移植者にLCMVが認められたが、各集団に認められたのは、単一かつ独自の株であった。どちらの集団でも、LCMVはドナーから検出されなかった。2005年のドナーは、自宅で飼っていたペットのハムスターと接触しており、このハムスターは、被移植者で検出されたものと同一のLCMV株に感染していた。一方、2003年の集団ではLCMV感染源は認められなかった。被移植者は、移植後3週間以内に、腹痛、精神状態の変容、血小板減少症、トランスアミナーゼ値上昇、凝固障害、移植臓器の機能不全及び発熱又は白血球増多症を引き起こした。下痢、術創周囲の発疹、腎不全、及び痙攣の発現は一定しなかった。被移植者8名のうち7名が、移植後9から76日の間に死亡し、リバビリン投与及び減量した免疫抑制療法を受けていた被移植者1名が生存した。

結論: 臓器移植によるLCMV感染の伝播が見られた2つの集団についてまとめた。

報告企業の意見	今後の対応
2003年12月及び2005年4月に固形臓器の移植を受けた2つの患者群でドナーからリンパ性脈絡髄膜炎ウイルスが伝播したと考えられたとの報告である。	今後も引き続き、新たなウイルス等による感染症の発生状況等に関する情報の収集に努める。

使用上の注意記載状況・その他参考事項等

合成血「日赤」
照射合成血「日赤」

血液を介するウイルス、細菌、原虫等の感染
vCJD等の伝播のリスク

ORIGINAL ARTICLE

Transmission of Lymphocytic Choriomeningitis Virus by Organ Transplantation

Staci A. Fischer, M.D., Mary Beth Graham, M.D., Matthew J. Kuehnert, M.D., Camille N. Kotton, M.D., Arjun Srinivasan, M.D., Francisco M. Marty, M.D., James A. Comer, Ph.D., Jeannette Guarner, M.D., Christopher D. Paddock, M.D., M.P.H.T.M., Dawn L. DeMeo, M.D., M.P.H., Wun-Ju Shieh, M.D., Ph.D., M.P.H., Bobbie R. Erickson, B.S., Utpala Bandy, M.D., M.P.H., Alfred DeMaria, Jr., M.D., Jeffrey P. Davis, M.D., Francis L. Delmonico, M.D., Boris Pavlin, M.D., Anna Likos, M.D., M.P.H., Martin J. Vincent, Ph.D., Tara K. Sealy, B.S., Cynthia S. Goldsmith, M.S., Daniel B. Jernigan, M.D., M.P.H., Pierre E. Rollin, M.D., Michelle M. Packard, M.P.H., Mitesh Patel, B.S., Courtney Rowland, B.S., Rita F. Helfand, M.D., Stuart T. Nichol, Ph.D., Jay A. Fishman, M.D., Thomas Ksiazek, D.V.M., Ph.D., Sherif R. Zaki, M.D., Ph.D., and the LCMV in Transplant Recipients Investigation Team*

ABSTRACT

BACKGROUND

In December 2003 and April 2005, signs and symptoms suggestive of infection developed in two groups of recipients of solid-organ transplants. Each cluster was investigated because diagnostic evaluations were unrevealing, and in each a common donor was recognized.

METHODS

We examined clinical specimens from the two donors and eight recipients, using viral culture, electron microscopy, serologic testing, molecular analysis, and histopathological examination with immunohistochemical staining to identify a cause. Epidemiologic investigations, including interviews, environmental assessments, and medical-record reviews, were performed to characterize clinical courses and to determine the cause of the illnesses.

RESULTS

Laboratory testing revealed lymphocytic choriomeningitis virus (LCMV) in all the recipients, with a single, unique strain of LCMV identified in each cluster. In both investigations, LCMV could not be detected in the organ donor. In the 2005 cluster, the donor had had contact in her home with a pet hamster infected with an LCMV strain identical to that detected in the organ recipients; no source of LCMV infection was found in the 2003 cluster. The transplant recipients had abdominal pain, altered mental status, thrombocytopenia, elevated aminotransferase levels, coagulopathy, graft dysfunction, and either fever or leukocytosis within three weeks after transplantation. Diarrhea, peri-incisional rash, renal failure, and seizures were variably present. Seven of the eight recipients died, 9 to 76 days after transplantation. One recipient, who received ribavirin and reduced levels of immunosuppressive therapy, survived.

CONCLUSIONS

We document two clusters of LCMV infection transmitted through organ transplantation.

From Rhode Island Hospital and Brown Medical School, Providence (S.A.F.); the Medical College of Wisconsin, Milwaukee (M.B.G.); the Divisions of Viral and Rickettsial Diseases (M.J.K., J.A.C., J.G., C.D.P., W.-J.S., B.R.E., B.P., A.L., M.J.V., T.K.S., C.S.G., P.E.R., M.M.P., M.P., C.R., R.F.H., S.T.N., T.K., S.R.Z.) and Healthcare Quality Promotion (A.S., D.B.J.), National Center for Infectious Diseases, Centers for Disease Control and Prevention, Atlanta; Massachusetts General Hospital and Harvard Medical School (C.N.K., F.L.D., J.A.F.) and Brigham and Women's Hospital and Harvard Medical School (F.M.M., D.L.D.) — all in Boston; the Rhode Island Department of Health, Providence (U.B.); the Massachusetts Department of Public Health, Boston (A.D.); the Wisconsin Department of Health and Family Services, Madison (J.P.D.); and the New England Organ Bank, Newton, Mass. (F.L.D.). Address reprint requests to Dr. Kuehnert at the Division of Viral and Rickettsial Diseases, National Center for Infectious Diseases, Centers for Disease Control and Prevention, 1600 Clifton Rd., Mailstop A-30, Atlanta, GA 30333, or at mkuehnert@cdc.gov.

Drs. Ksiazek and Zaki contributed equally to this article.

*The members of the Lymphocytic Choriomeningitis Virus (LCMV) in Transplant Recipients Investigation Team are listed in the Appendix.

N Engl J Med 2006;354:2235-49.

LYMPHOCYTIC CHORIOMENINGITIS VIRUS (LCMV) is a rodent-borne, Old World arenavirus that has been reported to cause asymptomatic or mild, self-limited illness in otherwise healthy humans. It is a known cause of aseptic meningitis, but fatal infection is rare.[1-4] Transmission of infection from a woman to a fetus can result in hydrocephalus, chorioretinitis, or microcephaly.[5-8] Outside of vertical transmission during pregnancy, human-to-human transmission of LCMV has not been described.[9] We describe two clusters of unexplained clinical syndromes in transplant recipients and the subsequent investigations to identify donor-transmitted infection as the cause of illness.

METHODS

CASE REPORTS

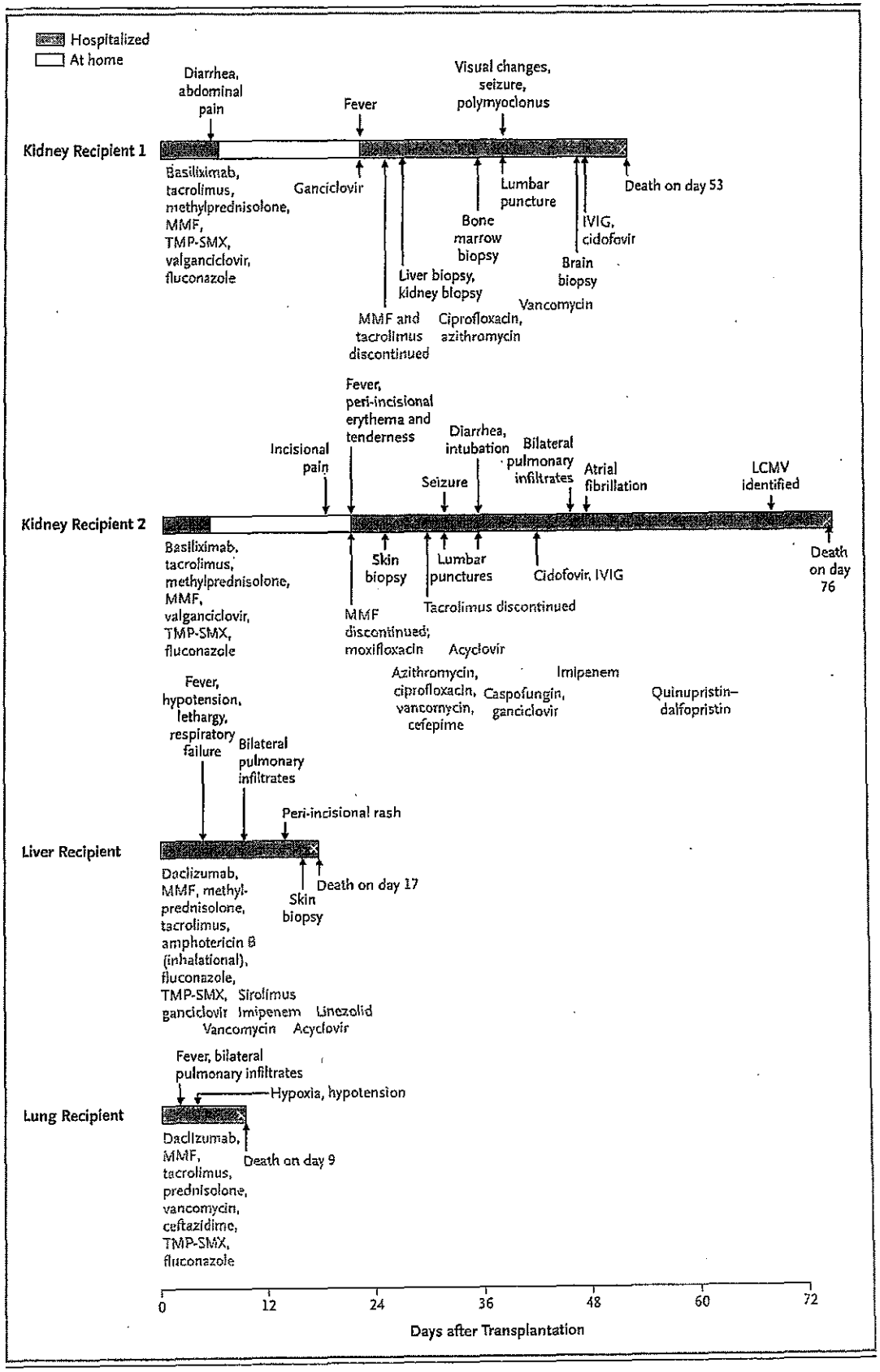
The 2003 Cluster

In December 2003, unexplained febrile illnesses developed in four recipients of solid organs from a common donor (Fig. 1; additional information on clinical symptoms and laboratory findings for each recipient is listed in Table 1 of the Supplementary Appendix, available with the full text of this article at www.nejm.org).[10] Kidney Recipient 1 was a 46-year-old man with diabetes. Diarrhea and mild, diffuse abdominal pain developed on post-transplantation day 5, but his condition was stable and he was discharged home the following day. He was readmitted on post-transplantation day 23 with fever, persistent watery diarrhea, and worsening abdominal pain. Laboratory studies revealed leukopenia with elevated aminotransferase and creatinine levels. Ganciclovir therapy was initiated because of concern about possible cytomegalovirus infection. Tacrolimus and mycophenolate mofetil were discontinued. Examination of kidney-, liver-, and bone marrow–biopsy specimens did not reveal an infectious cause. On day 40 after transplantation, seizures and polymyoclonus developed. The patient reported blurred vision, and chorioretinitis was noted on ophthalmologic examination. Cerebrospinal fluid studies revealed a markedly elevated level of protein (720 mg per deciliter), a normal glucose level (147 mg per deciliter [8.2 mmol per liter]), and 4 white cells and 3 red cells per cubic centimeter. Polymerase-chain-reaction (PCR) tests for cytomegalovirus, herpes simplex virus, varicella–zoster virus, Epstein–Barr virus, human herpesvirus 6, enterovirus, adenovirus, *Mycobacterium tuberculosis*, and *Borrelia burgdorferi* were negative. Magnetic resonance imaging of the brain revealed bilateral, hemispheric, subdural fluid collections and diffuse dural thickening. Examination of a specimen obtained by dural biopsy on post-transplantation day 47 showed fibrosis; cidofovir and intravenous immune globulin were initiated for the suspected presence of an unknown viral pathogen. The patient's condition continued to deteriorate, and he died on post-transplantation day 53. An autopsy revealed bronchopneumonia and hepatic congestion without inflammation.

Figure 1 (facing page). Clinical Course of Lymphocytic Choriomeningitis Virus (LCMV) Infection in the 2003 Cluster.

MMF denotes mycophenolate mofetil, TMP-SMX trimethoprim–sulfamethoxazole, IVIG intravenous immune globulin, and X death. Immunosuppressive agents are shown in red, and antimicrobial agents in blue.

Kidney Recipient 2 was a 56-year-old man with glomerulonephritis. He was discharged home on post-transplantation day 5 but was readmitted on post-transplantation day 22 with fever, leukopenia, and peri-incisional erythema and tenderness. A skin-biopsy specimen obtained at the wound edge revealed basal-cell vacuolation suggestive of viral infection; no viral inclusions were seen, and immunohistochemical stains were negative for cytomegalovirus and adenovirus. Tacrolimus and mycophenolate mofetil were discontinued. Altered mental status and seizures with myoclonus developed on post-transplantation day 31. Cerebrospinal fluid studies revealed a markedly elevated protein level (620 mg per deciliter), a low glucose level (57 mg per deciliter [3.2 mmol per liter]), and 12 white cells (48 percent lymphocytes and 22 percent monocytes) and 6 red cells per cubic centimeter. PCR testing for the same infectious agents as in the case of Kidney Recipient 1 was unrevealing. Magnetic resonance imaging showed subdural fluid collections with diffuse dural enhancement. The patient's condition continued to deteriorate, with photophobia, nuchal rigidity, diarrhea, thrombocytopenia, diffuse erythroderma, respiratory failure, and atrial fibrillation, despite empirical administration of cidofovir and intravenous immune globulin. He died on post-transplantation day 76; an autopsy revealed meningoencephalitis and acute bronchopneumonia.



The liver recipient was a 40-year-old woman with alcoholic cirrhosis whose early postoperative course was marked by fever, lethargy, and hypotension. Markedly elevated aminotransferase levels, leukopenia, and bilateral pulmonary infiltrates with respiratory failure developed. Liver biopsy revealed focal centrilobular coagulative necrosis. On post-transplantation day 14, a perincisional petechial rash was noted; skin biopsy revealed chronic inflammation and hemorrhage. She died on day 17 after transplantation. An autopsy revealed extensive hepatic necrosis without evidence of infection.

A common source of infection in these recipients was suspected, all of whom underwent transplantation at the same facility. Therefore, the Department of Public Health of the Wisconsin Department of Health and Family Services and the organ-procurement organization (OPO) coordinating the transplantations were notified for assistance.

The lung recipient, who had undergone transplantation at a different facility, was a 46-year-old man with chronic obstructive pulmonary disease who had been receiving prednisolone daily for three months before transplantation. Within four days after transplantation, hypotension, bilateral pulmonary infiltrates, and leukocytosis developed; broad-spectrum antimicrobial agents were administered. Fever (temperature, 38.3°C), hypoxia, and refractory hypotension ensued. He died on day 9 after transplantation. An autopsy revealed diffuse alveolar damage without evidence of rejection.

The donor was a 51-year-old man who had been found unresponsive, with apparent head trauma. Computed tomography (CT) of the brain revealed a large, right-sided subdural hematoma with a midline shift. There was no improvement in his neurologic status, and he was declared brain-dead on hospital day 2. He received no blood products and was afebrile throughout his hospitalization. Donor-eligibility screening and testing detected no infection precluding organ or tissue donation. His liver, lungs, kidneys, and multiple musculoskeletal and vascular tissues were recovered for transplantation. Tissue specimens from the donor and recipients were submitted to the Centers for Disease Control and Prevention (CDC) for additional testing (Table 1).

Table 1. Summary of Laboratory Evaluations for Lymphocytic Choriomeningitis Virus Infection in the 2003 Cluster.*

Patient	Outcome or Status	Immunohistochemical Staining	Serologic Testing		Culture
			IgM	IgG	
Donor†	No reported disease	–	–	–	–
Lung recipient‡	Death 9 days after transplantation	+	NT	NT	NT
Liver recipient§	Death 17 days after transplantation	+	NT	NT	NT
Kidney Recipient 1¶	Death 53 days after transplantation	+	–	–	+
Kidney Recipient 2‖	Death 76 days after transplantation	+	+	–	+

* NT denotes not tested.

† Specimens obtained at autopsy that tested negative included serum (serologic testing and virus isolation); bone marrow and blood vessel (virus isolation); and heart, stomach, tongue, thyroid gland, kidney, prostate, cerebral cortex, midbrain, pons, medulla, cerebellum, and spinal cord (immunohistochemical analysis).

‡ Specimens that tested positive by immunohistochemical analysis included lung, kidney, spleen, liver, and lymph node obtained at autopsy. No brain tissue was available for testing.

§ Specimens that tested positive by immunohistochemical analysis included skin obtained on day 15 and brain, lung, spleen, liver, heart, and adrenal gland obtained at autopsy.

¶ Specimens that tested positive included bone marrow, heart, kidney, liver, adrenal gland, and lung obtained at autopsy (immunohistochemical analysis); cerebrospinal fluid and a nasopharyngeal wash obtained on post-transplantation day 42 (viral culture); and leptomeninges in a brain-biopsy specimen obtained on post-transplantation day 47. Brain tissue obtained at autopsy tested negative.

‖ Specimens that tested positive included serum obtained on day 76 (serologic testing), skin on day 27 (immunohistochemical analysis), and blood on day 42 and cerebrospinal fluid on days 31 and 36 (viral culture). Bronchoalveolar-lavage fluid was positive on immunofluorescence assay. Brain tissue obtained at autopsy was negative.

The 2005 Cluster

Kidney Recipient A was a 48-year-old man admitted to a hospital in Rhode Island in late April 2005, 17 days after undergoing cadaveric renal transplantation (Fig. 2). He had been discharged home on post-transplantation day 7 with a creatinine level of 1.6 mg per deciliter (141 μmol per liter). At the time of readmission, he had had right-lower-quadrant pain in the area of the allograft for four or five days, as well as nausea, anorexia, diarrhea, fever, and chills. He was febrile (temperature, 38.7°C), and there was tender erythema over the area of the allograft, without incisional dehiscence or drainage. His creatinine level was 2.6 mg per deciliter (230 μmol per liter), and proteinuria, hematuria, a prolonged prothrombin time, and slightly elevated aminotransferase levels were present. Mycophenolate mofetil was discontinued, and administration of broad-spectrum antimicrobial agents was initiated. CT and ultrasonography of the abdomen and pelvis were unrevealing. Routine cultures of urine, blood, and stool were negative, as were studies of the stool for leukocytes, ova, parasites, *Clostridium difficile*, giardia, cryptosporidium, *Yersinia enterocolitica*, and rotavirus. Tacrolimus was discontinued because of concern about the worsening infection of uncertain cause. His temperature rose to 40.4°C, and he had copious diarrhea, dyspnea, and tender erythema extending from the area over the allograft to the right flank. Examination of biopsy specimens of the colon and kidney revealed no inflammation or viral inclusions.

Kidney Recipient B was a 54-year-old man who was admitted to the same hospital with fever. He had undergone cadaveric renal transplantation 17 days previously and had been discharged home on post-transplantation day 8 with a creatinine level of 3.3 mg per deciliter (292 μmol per liter). On post-transplantation day 14, mycophenolate mofetil was discontinued because of diarrhea. Fever and pain developed in the right lower quadrant over the area of the allograft, and he was readmitted for evaluation. He was lethargic and febrile (temperature, 38.4°C) and had tender erythema overlying the allograft, without incisional drainage. His creatinine level was 4.0 mg per deciliter (354 μmol per liter), with a platelet count of 113,000 per cubic millimeter and an alanine aminotransferase level of 298 IU per liter. Routine cultures of urine, blood, and stool were negative, and ultrasonography and CT of the abdomen and pelvis were unrevealing. Fever, diarrhea, and pain persisted, despite empirical use of broad-spectrum antimicrobial agents. A percutaneous liver biopsy was performed on post-transplantation day 23 because of worsening hepatitis and leukocytosis. Multiple foci of hepatocellular necrosis without inflammation or viral inclusions were noted. Later that day, he had a cardiac arrest and died. An autopsy revealed coronary artery disease and diffuse cerebral edema without meningitis or encephalitis.

A review of the records of the hospital's transplantation center revealed that the kidney recipients shared a common donor who had also been hospitalized there. The donor was a 45-year-old woman with hypertension who had presented to the emergency department with a five-day history of right-sided headache and acute left-sided weakness. She was alert and afebrile and had left-sided hemiparesis. CT of the brain revealed an infarct in the distribution of the right middle cerebral artery, and tissue plasminogen activator was administered. Throughout her hospitalization, she was afebrile and received no blood products or antimicrobial agents. She had a normal white-cell count on admission, with normal hepatic enzyme levels and platelet counts throughout her course. Intracerebral and subarachnoid hemorrhages with uncal herniation subsequently developed, and she was declared brain-dead. The donor met the screening criteria for organ and tissue donation, and surgical teams procured the lungs, kidneys, liver and associated blood vessels, skin, and corneas. Cultures of urine and blood performed at the time of organ procurement were negative. Examination of preimplantation biopsy specimens of the liver and kidney revealed no inflammation or granulomas. An autopsy revealed infarction in the distribution of the right middle cerebral artery, subarachnoid and left frontal intracerebral hemorrhages, and a patent foramen ovale. There was no evidence of infection.

The OPO coordinating the transplantations was contacted to obtain additional information about the donor. Physicians caring for the liver and lung recipients were contacted for information on their clinical status.

The liver recipient was a 54-year-old man with cirrhosis and chronic hepatitis B and C. In the initial days after the transplantation, he had head-

ache, fever (temperature, 39.1°C), and abdominal and right-shoulder pain. Leukopenia, thrombocytopenia, rising aminotransferase levels, and prolongation of the prothrombin time were noted. Administration of broad-spectrum antimicrobial agents was initiated; multiple cultures were negative. Exploratory laparotomy revealed intraabdominal hematoma without evidence of infection. The patient had a single, generalized seizure with hypotension and subsequent worsening of renal, hepatic, and respiratory function. No seizure focus was identified on radiologic imaging or electroencephalography. Examination of a liver-biopsy specimen revealed mild portal inflammation, liver-cell regeneration, cholestasis, and mild steatosis; these findings were interpreted as transplant-associated ischemia. Left bundle-branch block and atrial fibrillation developed. The patient became obtunded, with worsening coagulopathy and multiorgan failure. On post-transplantation day 21, high-dose methylprednisolone and antithymocyte globulin were administered for suspected acute graft rejection. Fever and hypotension persisted, with increases in aminotransferase and lactate dehydrogenase levels. The cause of his multiorgan failure was unclear. He had a cardiac arrest and died on post-transplantation day 26. An autopsy revealed extensive hepatic necrosis, bronchopneumonia, pulmonary edema, and subarachnoid hemorrhage.

The lung recipient was a 41-year-old man with cystic fibrosis. He was extubated on post-transplantation day 2 but became delirious the following day and had leukocytosis and thrombocytopenia. Chest radiographs revealed right-lower-lobe infiltrates. His temperature rose to 37.9°C, and diffuse abdominal pain and respiratory distress developed. CT of the chest on post-transplantation day 16 showed bilateral air-space disease, a finding interpreted as evidence of acute rejection, and high-dose methylprednisolone was administered. His creatinine level, prothrombin time, and aminotransferase levels steadily increased, and intermittent atrial fibrillation developed. On post-transplantation day 19, a pustular rash was noted on the face and trunk; examination of a skin-biopsy specimen revealed folliculitis, and aerobic, fungal, and viral cultures were negative. Symmetric effusions of the knees, elbows, and ankles developed. The hypoxemia and acidemia progressed, and the patient died on post-transplantation day 23. An autopsy revealed organizing diffuse alveolar damage and extensive geographic hepatic necrosis without inflammation or viral cytopathic changes.

Figure 2 (facing page). Clinical Course of Lymphocytic Choriomeningitis Virus (LCMV) Infection in the 2005 Cluster.

MMF denotes mycophenolate mofetil, TMP-SMX trimethoprim–sulfamethoxazole, CVVH continuous venous–venous hyperfiltration, and X death. Immunosuppressive agents are shown in red, and antimicrobial agents in blue.

Because of concern about transplant-transmitted infection in the organ recipients, the Rhode Island Department of Health, the Massachusetts Department of Public Health, and the CDC were contacted for assistance in investigating the causes of the recipients' illnesses. Causes considered included acute hepatitis A, leptospirosis, toxoplasmosis, enterovirus infection, and flavivirus infection. Tissue specimens from the donor and recipients were submitted to the CDC for additional testing.

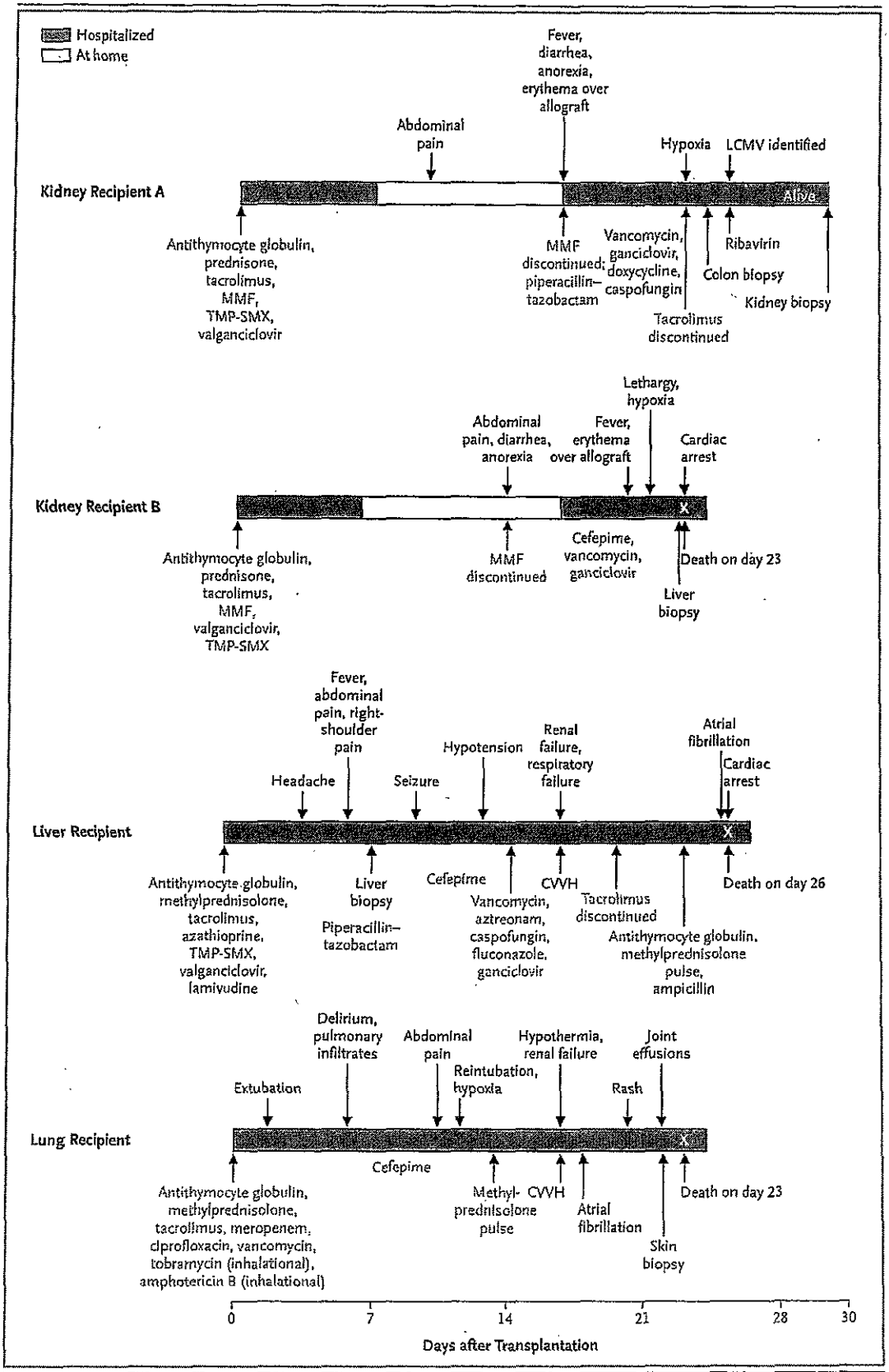
EPIDEMIOLOGIC INVESTIGATIONS

For both clusters, epidemiologic investigations were conducted at the transplantation centers and coordinating OPOs. For the 2005 cluster, epidemiologic investigations were also performed at the donor's home and workplace by state public health authorities and the CDC.

VIRUS ISOLATION AND INDIRECT FLUORESCENCE MICROSCOPY

Virus isolation was attempted by inoculation of Vero E6 cells with cerebrospinal fluid, serum, blood, and 10 percent fresh-tissue suspensions. Cultures were examined by thin-section electron microscopy. "Spot" slides of culture cells were also evaluated by indirect fluorescent antibody testing with the use of specific mouse hyperimmune ascitic fluids prepared against the Armstrong strain of LCMV.[11]

In the 2003 investigation, virus isolation was also attempted by inoculating suckling mice (*Mus musculus*) with fluid specimens (0.03 ml intracranially and 0.1 ml intraperitoneally) or with 10 percent homogenates of frozen tissue. Mice were killed by exposure to isoflurane, and tissues were fixed in 10 percent neutral buffered formalin and evaluated with the use of immunohistochemical stains for LCMV, as described below.



Studies in animals were performed at the CDC and were approved by the CDC Laboratory Animal Care and Use Committee. Animal research was conducted in compliance with the Animal Welfare Act and other federal statutes and regulations relating to animals and experiments involving animals, and it adhered to the principles stated in the *Guide for the Care and Use of Laboratory Animals*, by the U.S. National Research Council.

HISTOPATHOLOGICAL AND IMMUNOHISTOCHEMICAL ANALYSES

Multiple formalin-fixed, paraffin-embedded tissue specimens, including biopsy and autopsy tissues from the donors and recipients, were stained with hematoxylin and eosin and various immunohistochemical stains by means of an immunoalkaline phosphatase technique.[12] For both clusters, initial tissue specimens were evaluated with immunohistochemical stains for a number of viral agents, including flaviviruses, adenoviruses, and herpesviruses 1, 2, 3, and 5. In the investigation of the 2003 cluster, immunohistochemical testing for LCMV was performed after identification of this virus by culture, indirect fluorescent antibody testing, and electron microscopy. In the investigation of the 2005 cluster, immunohistochemical testing for LCMV was included in the initial evaluation of tissue specimens. The primary antibodies used for LCMV immunohistochemical detection included hyperimmune rabbit and mouse anti-LCMV antibodies specific for LCMV and an anti–Lassa virus monoclonal antibody reactive with both Lassa virus and LCMV.[13]

GENETIC DETECTION AND CHARACTERIZATION OF VIRUS

RNA was extracted from clinical specimens or viral isolates, and specific molecular targets were amplified by reverse-transcriptase–PCR (RT-PCR) assays with the use of broadly reactive polymerase gene–specific primers for the detection of arenavirus RNA. The resulting complementary DNA products were purified, and nucleotide sequences were determined and analyzed. In the investigation of the 2005 cluster, after LCMV-specific sequences had been obtained from PCR products amplified from clinical specimens, a more sensitive, LCMV-specific, quantitative real-time RT-PCR (TaqMan) technology was developed and used for more extensive analysis of specimens.

Figure 3 (facing page). Pathological Studies Revealing Lymphocytic Choriomeningitis Virus (LCMV) in the 2003 Cluster.

Panel A, an electron micrograph of LCMV isolated in Vero E6 cells from the cerebrospinal fluid of Kidney Recipient 1, shows highly pleomorphic, 50-to-300-nm virions containing electron-dense particles. Panel B shows immunohistochemical staining of LCMV (red) in neurons and choroid-plexus ependymal cells of a mouse inoculated with the virus (immunoalkaline phosphatase with naphthol–fast red and hematoxylin counterstain; monoclonal anti-LCMV antibody). Panel C shows immunohistochemical staining of LCMV antigens in lung tissue from the lung recipient (monoclonal anti-LCMV antibody). The image in Panel D reveals extensive hepatocellular necrosis with minimal inflammatory-cell infiltrates in the liver recipient (hematoxylin and eosin). Panel E shows immunohistochemical staining of viral antigens in the transplanted liver (monoclonal anti–Lassa virus antibody). Panel F shows immunohistochemical staining of LCMV antigens in the donor kidney of Kidney Recipient 1 (monoclonal anti-LCMV antibody). Panel G shows immunohistochemical staining of viral antigens in the skin of Kidney Recipient 2 (monoclonal anti–Lassa virus antibody). All micrographs are shown at low magnification.

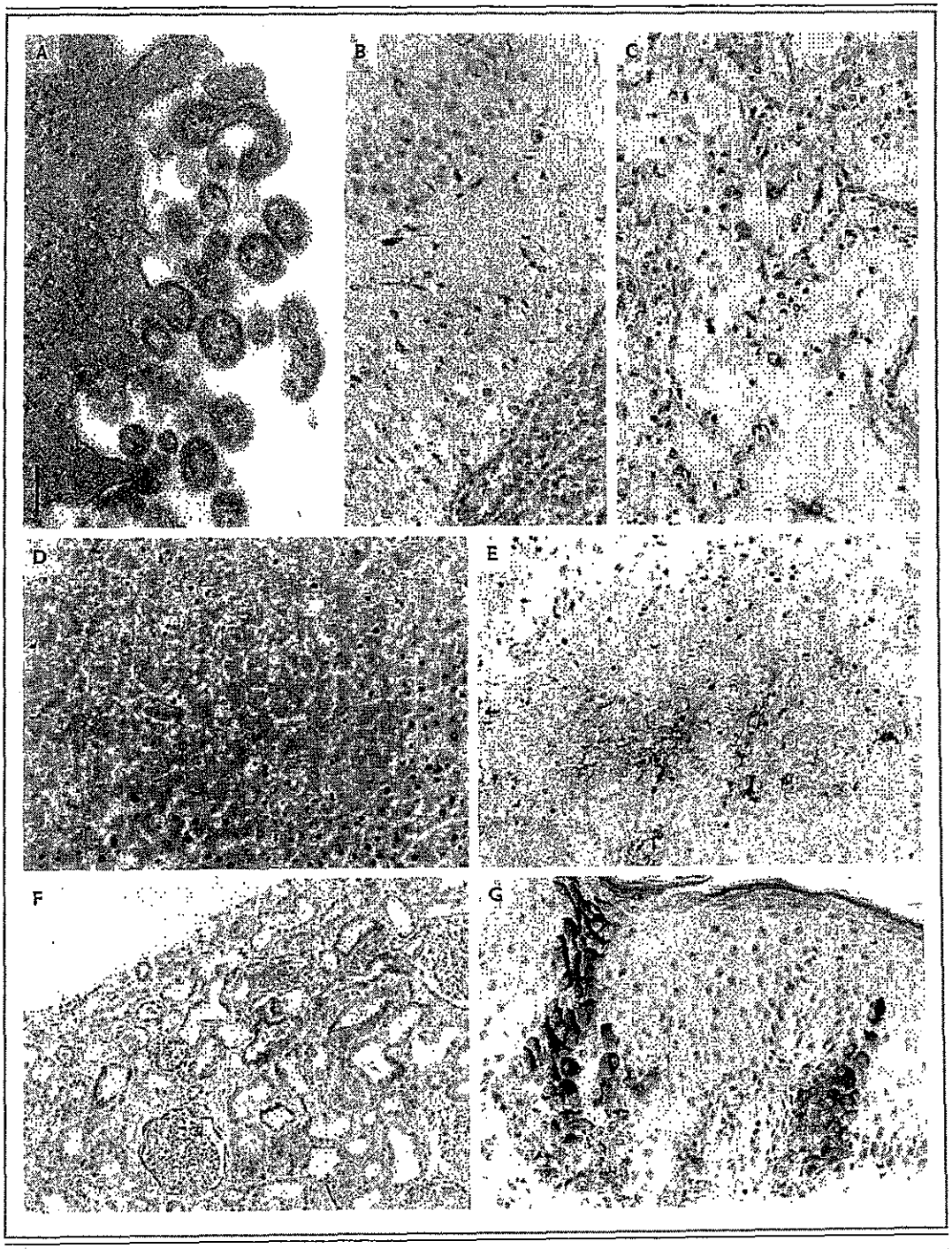
SEROLOGIC TESTING

An enzyme-linked immunosorbent assay was used to detect LCMV-specific IgM and IgG. The assay was performed as previously described,[11] with some modifications in commercially available components, such as microtiter plates and conjugates.

RESULTS

INVESTIGATION OF THE 2003 CLUSTER

Results of testing in the 2003 cluster are summarized in Figure 3 and Table 1. Electron microscopy of Vero E6 cell cultures inoculated with cerebrospinal fluid from Kidney Recipient 1 revealed viral particles compatible with an Old World arenavirus. Indirect fluorescent antibody testing of these cultures confirmed the identity of the arenavirus as LCMV. Subsequently, LCMV was identified by immunohistochemical analysis of the brain tissue of mice inoculated with cerebrospinal fluid from the same patient. LCMV was identified in multiple tissues from each of the four recipients. Nucleotide analysis of PCR products revealed that the viral isolates obtained from the two kidney recipients were identical and were distinct from previously described LCMV strains (Fig. 1 of the Supplementary Appendix). Extensive



testing of multiple donor tissues by means of immunohistochemical analysis, cell culture, and RT-PCR revealed no evidence of LCMV. Neither IgM nor IgG antibodies against LCMV were detected in the donor's serum. Interviews with the donor's family revealed no known rodent exposures. Investigation of procedures, materials, and personnel in hospitals at which the donor and recipients had received care revealed no likely route of LCMV transmission.

INVESTIGATION OF THE 2005 CLUSTER

Results of testing in the 2005 cluster are summarized in Figure 4 and Table 2 (and Fig. 1 of the

Supplementary Appendix). LCMV was detected by immunohistochemical staining, cell culture, and quantitative real-time RT-PCR in multiple tissues from all four recipients. Both kidney recipients had IgM antibodies reactive to LCMV. Extensive testing of multiple donor tissues revealed no evidence of LCMV. No IgM or IgG antibodies against LCMV were detected in the serum of the donor.

The epidemiologic investigation revealed that a member of the donor's household had brought home a pet hamster three weeks before the donor died. Although the donor had not been the primary caretaker of the hamster, she had had contact with the rodent's environment on multiple occasions. Her home and work environment contained no evidence of active rodent infestation. Testing of multiple hamster tissues by immunohistochemical analysis, quantitative real-time RT-PCR, and viral culture detected evidence of LCMV infection (Fig. 4 and Table 2). The primary caretaker of the hamster was asymptomatic but had LCMV-reactive IgG and IgM antibodies present in the serum. Nucleotide analysis of PCR products identified that the viral isolates from all four organ recipients and the hamster were identical, but that they differed from the strain identified in the 2003 cluster and previously described LCMV strains (Fig. 1 of the Supplementary Appendix).

After identification of LCMV as the etiologic agent, intravenous ribavirin (a loading dose of 30 mg per kilogram of body weight, followed by 16 mg per kilogram every six hours for four days and then 8 mg per kilogram every eight hours) was initiated in Kidney Recipient A beginning on post-transplantation day 26 (Fig. 2 of the Supplementary Appendix).[14] The fever and diarrhea decreased; the pain, tenderness, and erythema in the area of the allograft diminished; and the hypoxemia, elevation of aminotransferase levels, thrombocytopenia, and coagulopathy decreased. After the patient's clinical condition had stabilized, ribavirin administration was changed to the oral route (400 mg every morning and 600 mg every evening), and it was discontinued after a renal-biopsy specimen was found to be LCMV-negative by RT-PCR and immunohistochemical staining and after serum IgM became detectable, 63 days after transplantation. During 37 days of ribavirin treatment, clinically significant hemolytic anemia developed and required the transfusion of 19 units of packed red cells. Three hundred eleven days after transplantation, the patient had stable graft function without evidence of infection, after he had restarted immunosuppressive therapy with tacrolimus, mycophenolate mofetil, and prednisolone.

Figure 4 (facing page). Immunohistochemical Staining for Lymphocytic Choriomeningitis Virus (LCMV) in Tissue Samples from the Donor, the Donor's Household Hamster, and Organ Recipients in the 2005 Cluster.

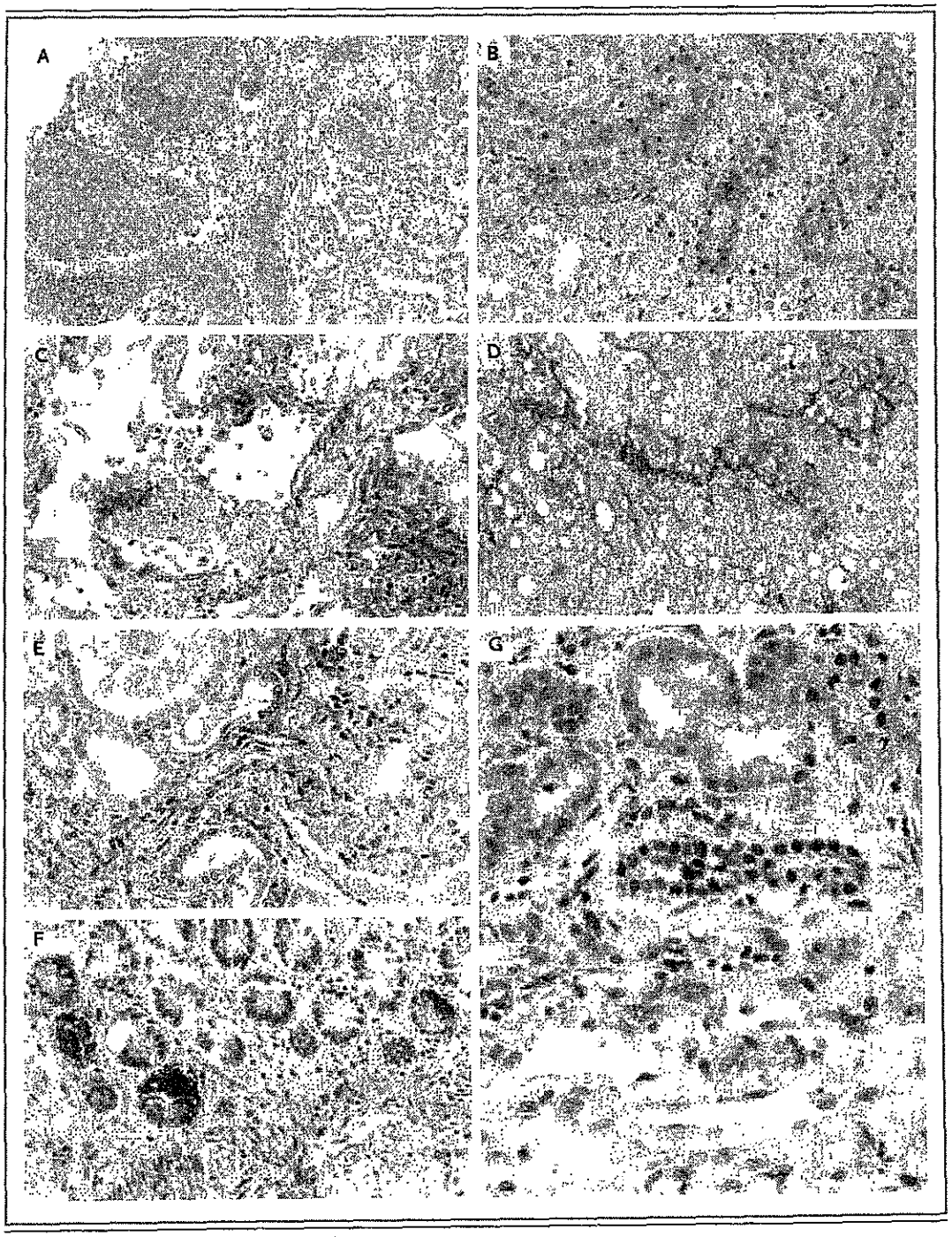
Red staining indicates the presence of LCMV antigens. The image in Panel A contains no immunohistochemical evidence of LCMV in choroid plexus from the donor. Panel B shows antigens in the kidney tubules of the donor's household hamster. Panel C shows LCMV antigens in lung tissue obtained at autopsy from the lung recipient; there are extensive hyaline-membrane formation and viral antigens in the interstitium. Panel D shows LCMV antigens in liver tissue obtained at autopsy from the liver recipient; viral antigens delineate the hepatocyte cytoplasmic membrane. Panel E shows LCMV antigens in a kidney specimen obtained at autopsy from Kidney Recipient B; viral antigens in endothelial cells are entering and exiting the glomerulus. Panel F shows LCMV antigens in a colon sample obtained at autopsy from Kidney Recipient B, with viral antigens in the muscularis mucosae and mucous cells of colonic glands. Panel G shows LCMV antigens in a kidney-biopsy specimen from Kidney Recipient A, who survived; viral antigens are in endothelial cells of the renal interstitium. (The studies shown in Panels A, B, E, and G used a rabbit anti-LCMV antibody, those in Panels C and D a mouse anti–Lassa virus antibody, and that in Panel F a mouse ascitic-fluid anti-LCMV antibody in an immunohistochemical assay with naphthol–fast red substrate and hematoxylin counterstain.) All micrographs are shown at low magnification.

TISSUE DISPOSITION

In the 2003 cluster, no additional tissues from the donor were transplanted. In the 2005 cluster, the corneas were transplanted into a 4-year-old girl and a 29-year-old woman in Algeria, neither of whom required systemic immunosuppression. Two hundred thirteen days after transplantation, neither patient had reported symptoms of infection or graft loss. The skin and liver-associated blood vessels were not transplanted.

DISCUSSION

We describe the transmission of LCMV by solid-organ transplantation. In both clusters, disseminated infection developed in the recipients of organs from a common donor who had no clini-



cal or laboratory evidence of infection. In the 2003 cluster, no rodent exposures could be identified, whereas the 2005 cluster was associated with recent donor exposure to an LCMV-infected pet hamster. The isolation of identical virus strains from the two kidney recipients in the 2003 cluster and from all the organ recipients and the donor household's pet hamster in the 2005 cluster indicates that in both clusters, LCMV was transmitted through organ transplantation.[15]

LCMV infection in humans is sporadic, is generally benign, and can be asymptomatic.[4,16,17] Serologic surveys suggest that up to 5 percent of adults in the United States have been infected with LCMV.[18,19] Humans become infected with LCMV by direct contact with rodents or through

Table 2. Summary of Laboratory Evaluations for Lymphocytic Choriomeningitis Virus Infection in the 2005 Cluster.*

Patient or Source of Specimen	Outcome or Status	Immunohistochemical Staining	Quantitative Real-Time RT-PCR†	Blood and Serum Testing		Culture
				IgM	IgG	
Donor‡	No reported disease	–	–	–	–	–
Liver recipient§	Death 26 days after transplantation	+	+	–	–	+
Lung recipient¶	Death 23 days after transplantation	+	+	–	–	+
Kidney Recipient B‖	Death 23 days after transplantation	+	+	+	–	+
Kidney Recipient A**	Survival	+	+	+	–	+
Hamster in donor's household††	No reported disease	+	+	NT	–	+
Hamster's caregiver‡‡	No reported symptoms	NA	–	+	+	–

* RT-PCR denotes reverse-transcriptase polymerase chain reaction, NT not tested, and NA not applicable.
† Quantitative real-time RT-PCR (TaqMan) assays were carried out as follows: complementary DNA was synthesized with a High Capacity cDNA Archive Kit (Applied Biosystems) and used as a template in a second reaction involving TaqMan Universal PCR Master Mix (Applied Biosystems), forward primer 5′TGGGACTCCATTCGCTATGG3′ at 1 μM per reaction, reverse primer 5′TTAAGTGCAAGGAACCACATCAA at 1 μM per reaction, and probe FAM 5′TGGTCAGCAATCGTGTCTATTGTTTCACAAA at 0.1 μM per reaction. Samples with a cycle-threshold value below 40 were considered positive.
‡ Autopsy specimens that tested negative by one or more tests included central nervous system tissue, heart, spleen, liver, pancreas, uterus, thyroid, gastrointestinal tract, muscle, skin, kidney, blood, and serum.
§ Autopsy specimens that tested positive by one or more tests included central nervous system tissue, lung, heart, kidney, skin, liver, spleen, gastrointestinal tract, adrenal gland, pancreas, testis, blood, and serum. Liver- and colon-biopsy specimens obtained on days 16 and 19, respectively, were also positive by immunohistochemical analysis.
¶ Autopsy specimens that tested positive by one or more tests included central nervous system tissue, lung, heart, kidney, skin, liver, spleen, gastrointestinal tract, blood, and serum.
‖ Autopsy specimens that tested positive by one or more tests included spleen, heart, lung, kidney, gastrointestinal tract, pancreas, liver, blood, and serum. Blood and serum samples obtained on day 17 were positive by PCR, serologic testing for IgM, and culture.
** A colon-biopsy specimen obtained on day 26 was positive, a kidney-biopsy specimen on day 31 was positive, and a kidney-biopsy specimen on day 56 was negative by immunohistochemical analysis. Serial blood or serum samples obtained on days 16 to 50 were positive, blood and serum obtained on days 52 and 85 were negative, urine obtained on days 24 and 37 was positive, urine obtained on day 85 was negative, and a kidney-biopsy specimen obtained on day 55 was negative by PCR and culture. Serial blood and serum samples obtained on days 16 to 39 were IgM negative, on days 52 and 85 IgM positive, and on days 16 to 85 IgG negative by serologic testing.
†† Necropsy specimens that were positive on one or more tests included urinary bladder, skin, muscle, testis, central nervous system tissue, heart, lung, kidney, adrenal gland, salivary gland, pancreas, liver, and spleen.
‡‡ Blood and serum specimens were evaluated.

aerosolized droplets from rodent secretions and excretions, including urine or feces.[20,21] Reports of sporadic infection have most frequently implicated the common house mouse, although outbreaks of infection have been reported after exposure to infected pet hamsters and laboratory rodents.[17,20-28]

In clinically apparent infection, the incubation period is 5 to 13 days, with subsequent fever, headache, and myalgias. Abdominal pain, diarrhea, and rash have been described.[16,20,22,26,29] A second phase of illness may be seen five to nine days after convalescence, with meningitis or, rarely, encephalomyelitis, orchitis, parotitis, pneumonitis, arthritis, myocarditis, or alopecia.[16,22,30] Recognized LCMV infection carries a mortality rate of less than 1 percent.[3]

The marked severity of LCMV-related illness in transplant recipients is probably the result of intensive immunosuppression, including T-cell depletion, coincident with direct viral inoculation by way of the transplanted organs. There are few data on the clinical behavior and outcomes of LCMV infection in immunocompromised patients. However, three patients with advanced lymphoma experimentally inoculated with the virus in a trial investigating its antitumor effect died with disseminated infection within 14 to 45 days after inoculation[31] — a pattern similar to that seen in the current clusters after solid-organ transplantation.

The clinical presentation of LCMV infection in the recipients was variable and included fever, diarrhea, peri-incisional erythema and tenderness, altered mental status, and respiratory insufficiency (as noted in the table of the Supplementary Appendix). Leukopenia or leukocytosis, thrombocytopenia, coagulopathy, renal insuf-

ficiency, and progressive liver dysfunction dominated the laboratory findings. Histopathological findings in all the recipients were characterized by necrotic and occasionally hemorrhagic foci in multiple tissues, with a notable absence of inflammatory infiltrates and viral inclusions. LCMV antigens present in some tissues (e.g., the gastrointestinal tract and skin) correlated with clinical symptoms (e.g., diarrhea and erythema or pustular rash, respectively). Antigens were identified in the leptomeninges of some patients in both clusters. However, signs of meningeal inflammation, though prominent in the 2003 cluster, were generally absent in the 2005 cluster. Clinical manifestations and pathological findings in all the cases were probably altered by immunosuppression.

To our knowledge, there have been no trials of antiviral agents in human LCMV disease. Ribavirin has demonstrated efficacy in the treatment of Lassa fever and possesses in vitro activity against LCMV infection, which prompted its use in the renal-transplant recipient who survived.[14,32] The role of ribavirin in this patient's improvement is unclear, since the level of immunosuppression was also considerably reduced, as it was for all four kidney recipients in the two clusters.

Rapid evaluation of organ-donor suitability is essential in transplantation to minimize the duration of ischemia and to preserve allograft function. Therefore, assays used to screen potential donors for transmissible infections must be rapid, sensitive, reproducible, and readily available to OPOs. The Food and Drug Administration has not approved any diagnostic tests for LCMV infection. Furthermore, the sensitivity of currently available assays is not adequate for routine donor screening, as demonstrated by the negative results of tests on a wide array of clinical specimens from the donors in both clusters. The use of information pertaining to recent rodent exposure for donor-suitability screening may exclude healthy donors from an already limited organ-donor pool. However, the collection of additional epidemiologic information on donors' exposures may be useful, notably for the investigation of unusual outcomes after transplantation. Zoonotic-disease transmission after transplantation is also a concern; immunosuppressed persons should take special care and limit exposure to some animals, including certain pets (additional information is available from the CDC at www.cdc.gov/healthypets).

Transplant recipients are susceptible to infection with a variety of donor-derived pathogens, including West Nile virus, *Trypanosoma cruzi*, rabies virus, and now LCMV.[33-36] Although such infections are probably uncommon, outcomes can be fatal, and diagnosis is feasible with specialized laboratory testing. Diagnosis of LCMV infection is usually made by serologic testing, isolation of the virus from the blood or cerebrospinal fluid, or PCR testing.[37-39] Because immunohistochemical staining revealed LCMV in multiple biopsy specimens obtained to evaluate unexplained post-transplantation symptoms in the clusters described in the current report, such testing might be beneficial in the early diagnosis of LCMV infection.

Each year, approximately 25,000 organ transplantations are performed at more than 250 transplantation centers throughout the United States.[40] Allocation policies commonly result in the distribution of organs from a single donor to multiple transplantation centers. It is unlikely that either LCMV-illness cluster would have been identified without the allocation of kidneys to two recipients in whom similar symptoms simultaneously developed after undergoing transplantation at the same hospital. Similar chance clinical observations have been critical in the recognition of recent transplant-associated outbreaks of rabies and West Nile virus infection.[33,35]

The Organ Procurement and Transplantation Network (OPTN), which is operated by the United Network for Organ Sharing, requires transplantation centers to report certain outcomes, including allograft failure and the death of transplant recipients, in a timely manner. In April 2005, the OPTN revised its policies to require the reporting of suspected donor-transmitted medical conditions (including cancers and infections) to the procuring OPO, which is then responsible for investigating and communicating with the transplantation centers caring for the recipients of other transplants from the donor and the involved tissue and eye banks.[41]

Investigation of potential donor-transmitted infection requires rapid communication among physicians in multiple transplantation centers, OPOs, and public health authorities. An immediate system for tracking and disseminating pertinent patient data is needed. Until such a system can be established, clinicians must recognize that the presence of an unusual constellation of

symptoms, particularly during the first few weeks after transplantation, should raise the possibility of donor-transmitted infection. Prompt notification of the OPO and public health authorities can help facilitate rapid investigation and discovery of these events.

Dr. Kotton reports having received grant support from Wyeth and the Massachusetts General Hospital Clafin Award. Dr. Fishman reports having received consulting fees or lecture fees from Astellas, Enzon, Novartis, Pfizer, and Roche. No other potential conflict of interest relevant to this article was reported.

The views expressed herein are those of the authors and not necessarily those of the Department of Health and Human Services.

We are indebted to Jennifer Betts, Kimberly Slaughter, Deborah Cannon, and Thomas Stevens for specimen processing, serologic testing, and virus isolation and characterization; to Nicole Lundstrom and Sherry Hayes for manuscript preparation; and to Donita Croft, James Kazmierczak, Mark Sotir, and Mark Wegner for investigation assistance.

APPENDIX

The members of the LCMV in Transplant Recipients Investigation Team are as follows: *Brigham and Women's Hospital:* D.A. Milner and M. Wilck; *Centers for Disease Control and Prevention:* C. Albarino, B. Amman, M. Bell, B. Bird, B. Holloway, J. Mills, and J. Towner; *Massachusetts Department of Public Health:* C. Daniel, S. Fleming, P. Kludt, and B.T. Matyas; *Massachusetts General Hospital:* R. Chung, A.B. Cosimi, N. Elias, N. Goes, M. Hertl, A. Reid, E.S. Rosenberg, and R.N. Smith; *Medical College of Wisconsin:* C.P. Johnson, S.C. Kehl, and C. Schultzenberg; *New England Organ Bank:* R.S. Luskin and K.J. O'Connor; *Rhode Island Department of Health:* C. Hannafin and C. Vanner; and *Rhode Island Hospital:* R. DeLellis, A. Gautam, R. Gohh, K. Kurek, P. Morrissey, and A. Yango.

REFERENCES

1. Roebroek RM, Postma BH, Dijkstra UJ. Aseptic meningitis caused by the lymphocytic choriomeningitis virus. Clin Neurol Neurosurg 1994;96:178-80.
2. Deibel R, Schryver GD. Viral antibody in the cerebrospinal fluid of patients with acute central nervous system infections. J Clin Microbiol 1976;3:397-401.
3. Warkel RL, Rinaldi CF, Bancroft WH, Cardiff RD, Holmes GE, Wilsnack RE. Fatal acute meningoencephalitis due to lymphocytic choriomeningitis virus. Neurology 1973;23:198-203.
4. Peters CJ. Arenavirus diseases. In: Porterfield JS, ed. Exotic viral infections. London: Chapman & Hall Medical, 1995:227-46.
5. Bechtel RT, Haught KA, Mets MB. Lymphocytic choriomeningitis virus: a new addition to the TORCH evaluation. Arch Ophthalmol 1997;115:680-1.
6. Barton LL, Mets MB, Beauchamp CL. Lymphocytic choriomeningitis virus: emerging fetal teratogen. Am J Obstet Gynecol 2002;187:1715-6.
7. Wright R, Johnson D, Neumann M, et al. Congenital lymphocytic choriomeningitis virus syndrome: a disease that mimics congenital toxoplasmosis or cytomegalovirus infection. Pediatrics 1997;100:E9.
8. Enders G, Varho-Göbel M, Löhler J, Terletskaia-Ladwig E, Eggers M. Congenital lymphocytic choriomeningitis virus infection: an underdiagnosed disease. Pediatr Infect Dis J 1999;18:652-5.
9. Biggar RJ, Douglas RG, Hotchin J. Lymphocytic choriomeningitis associated with hamsters. Lancet 1975;1:856-7.
10. Paddock C, Ksiazek T, Comer JA, et al. Pathology of fatal lymphocytic choriomeningitis virus infection in multiple organ transplant recipients from a common donor. Mod Pathol 2005;18:Suppl 1:263A. abstract.
11. Ksiazek TG, Erdman D, Goldsmith CS, et al. A novel coronavirus associated with severe acute respiratory syndrome. N Engl J Med 2003;348:1953-66.
12. Zaki SR, Greer PW, Coffield LM, et al. Hantavirus pulmonary syndrome: pathogenesis of an emerging infectious disease. Am J Pathol 1995;146:552-79.
13. Puglielli MT, Browning JL, Brewer AW, et al. Reversal of viral-induced systemic shock and respiratory failure by blockade of the lymphotoxin pathway. Nat Med 1999;5:1370-4.
14. McCormick JB, King IJ, Webb PA, et al. Lassa fever: effective therapy with ribavirin. N Engl J Med 1986;314:20-6.
15. Lymphocytic choriomeningitis virus infection in organ transplant recipients — Massachusetts, Rhode Island, 2005. MMWR Morb Mortal Wkly Rep 2005;54:537-9.
16. Rousseau MC, Saron MF, Brouqui P, Bourgeade A. Lymphocytic choriomeningitis virus in southern France: four case reports and a review of the literature. Eur J Epidemiol 1997;13:817-23.
17. Baum SG, Lewis AM, Rowe WP, Huebner RJ. Epidemic nonmeningitic lymphocytic-choriomeningitis-virus infection: an outbreak in a population of laboratory personnel. N Engl J Med 1966;274:934-6.
18. Stephensen CB, Blount SR, Lanford RE, et al. Prevalence of serum antibodies against lymphocytic choriomeningitis virus in selected populations from two U.S. cities. J Med Virol 1992;38:27-31.
19. Childs JE, Glass GE, Ksiazek TG, Rossi CA, Oro JG, Leduc JW. Human-rodent contact and infection with lymphocytic choriomeningitis and Seoul viruses in an inner-city population. Am J Trop Med Hyg 1991;44:117-21.
20. Biggar RJ, Woodall JP, Walter PD, Haughie GE. Lymphocytic choriomeningitis outbreak associated with pet hamsters: fifty-seven cases from New York State. JAMA 1975;232:494-500.
21. Dykewicz CA, Dato VM, Fisher-Hoch SP, et al. Lymphocytic choriomeningitis outbreak associated with nude mice in a research institute. JAMA 1992;267:1349-53. [Erratum, JAMA 1992;268:874.]
22. Vanzee BE, Douglas RG, Betts RF, Bauman AW, Fraser DW, Hinman AR. Lymphocytic choriomeningitis in university hospital personnel: clinical features. Am J Med 1975;58:803-9.
23. Biggar RJ, Schmidt TJ, Woodall JP. Lymphocytic choriomeningitis in laboratory personnel exposed to hamsters inadvertently infected with LCM virus. J Am Vet Med Assoc 1977;171:829-32.
24. Gregg MB. Recent outbreaks of lymphocytic choriomeningitis in the United States of America. Bull World Health Organ 1975;52:549-53.
25. Bowen GS, Calisher CH, Winkler WG, et al. Laboratory studies of a lymphocytic choriomeningitis virus outbreak in man and laboratory animals. Am J Epidemiol 1975;102:233-40.
26. Hirsch MS, Moellering RC Jr, Pope HG, Poskanzer DC. Lymphocytic-choriomeningitis-virus infection traced to a pet hamster. N Engl J Med 1974;291:610-2.
27. Maetz HM, Sellers CA, Bailey WC, Hardy GE Jr. Lymphocytic choriomeningitis from pet hamster exposure: a local public health experience. Am J Public Health 1976;66:1082-5.
28. Skinner HH, Knight EH. The potential role of Syrian hamsters and other small animals as reservoirs of lymphocytic choriomeningitis virus. J Small Anim Pract 1979;20:145-61.
29. Armstrong D, Fortner JG, Rowe WP, Parker JC. Meningitis due to lymphocytic choriomeningitis virus endemic in a hamster colony. JAMA 1969;209:265-7.
30. Lewis JM, Utz JP. Orchitis, parotitis and meningoencephalitis due to lymphocytic-choriomeningitis virus. N Engl J Med 1961;265:776-80.
31. Horton J, Hotchin JE, Olson KB, Davies

JNP. The effects of MP virus infection in lymphoma. Cancer Res 1971;31:1066-8.
32. Enria DA. Maiztegui JI. Antiviral treatment of Argentine hemorrhagic fever. Antiviral Res 1994;23:23-31.
33. Iwamoto M, Jernigan DB, Guasch A, et al. Transmission of West Nile virus from an organ donor to four transplant recipients. N Engl J Med 2003;348:2196-203.
34. West Nile infections in organ transplant recipients — New York and Pennsylvania, August–September 2005. MMWR Morb Mortal Wkly Rep 2005;54:1021-3.
35. Srinivasan A, Burton EC, Kuehnert MJ, et al. Transmission of rabies virus from an organ donor to four transplant recipients. N Engl J Med 2005;352:1103-11.
36. Chagas disease after organ transplantation — United States, 2001. MMWR Morb Mortal Wkly Rep 2002;51:210-2.
37. Lehmann-Grube F, Kallay M, Ibscher B, Schwartz R. Serologic diagnosis of human infections with lymphocytic choriomeningitis virus: comparative evaluation of seven methods. J Med Virol 1979;4:125-36.
38. Park JY, Peters CJ, Rollin PE, et al. Development of a reverse transcription-polymerase chain reaction assay for diagnosis of lymphocytic choriomeningitis virus infection and its use in a prospective surveillance study. J Med Virol 1997;51:107-14.
39. Besselsen DG, Wagner AM, Loganbill JK. Detection of lymphocytic choriomeningitis virus by use of fluorogenic nuclease reverse transcriptase-polymerase chain reaction analysis. Comp Med 2003;53:65-9.
40. OPTN/SRTR 2004 annual report. Richmond, Va.: Organ Procurement and Transplantation Network, United Network for Organ Sharing, 2004. (Accessed May 1, 2006, at http://www.optn.org/data/annualReport.asp.)
41. Acquired immune deficiency syndrome (AIDS), human pituitary derived growth hormone (HPDGH), and reporting of potential recipient diseases or medical conditions, including malignancies, of donor origin. Richmond, Va.: Organ Procurement and Transplantation Network, United Network for Organ Sharing, 2004. (Accessed May 1, 2006, at http://www.optn.org/PoliciesandBylaws/policies/docs/policy_16.doc.)

医薬品 研究報告 調査報告書

識別番号・報告回数			報告日	第一報入手日	新医薬品等の区分	機構処理欄
				2006. 8. 22	該当なし	

一般的名称	洗浄人赤血球浮遊液	研究報告の公表状況	AABB Weekly Report. 2006 Jul 21.	公表国 アイルランド
販売名(企業名)	洗浄赤血球「日赤」(日本赤十字社) 照射洗浄赤血球「日赤」(日本赤十字社)			

研究報告の概要

○アイルランド輸血サービスで、プリオン除去フィルターの治験中止
アイルランド輸血サービスは、クロイツフェルト・ヤコブ病(CJD)の病因となるプリオンを供血血液から除去する新しいフィルターを1年間使用した後、治験の中止を決定した。7月11日付Irish Examinerの記事によると、輸血サービスは昨年フィルターシステムを購入したが、十分な効果が得られず、CJDは検出されずに供血血液に混入し続けている疑いがあるとして、使用を中止した。

報告企業の意見	今後の対応
アイルランド輸血サービスが、プリオン除去フィルターの試験の中止を決定したとの報告である。	今後も引き続き、プリオン除去フィルターなどプリオン病に関する新たな知見及び情報の収集に努める。

使用上の注意記載状況・その他参考事項等

洗浄赤血球「日赤」
照射洗浄赤血球「日赤」

血液を介するウイルス、細菌、原虫等の感染
vCJD等の伝播のリスク

According to a July 14 *Modern Healthcare Today* article, the Bush administration's nominee for deputy treasury secretary for tax policy has also agreed to revisit a longstanding IRS rule on community benefits, known as Revenue Ruling 69-545.

Science and Medicine

The first observational study to assess the progress of patients in the U.K. undergoing a controversial treatment for variant Creutzfeldt-Jakob disease (vCJD) has indicated that the drug may be beneficial in extending the life of sufferers. According to a July 12 *Guardian* article, Pentosan, or PPS, injected into the skull around the brain and administered during neurosurgery each month has resulted in prolonging the lives of patients of all ages. The treatment works by slowing the loss of brain tissue, but it does not halt it. Although eight patients in the U.K. have been given PPS, health officials do not officially sanction the drug, and some have expressed concern that it could cause the brain to hemorrhage.

Ian Bone, the Glasgow neurologist who led the study, noted that "the patients treated with PPS appear to have survived for unusually long periods." Bone cautioned, however, that "we cannot conclude with certainty that the treatment has a beneficial effect because it is impossible to make direct comparison with similar but untreated patients."

International

Europe's largest homosexual news organization, *Pink News*, recently reported that Russia may soon lift its ban on blood donations from homosexual donors. According to a July 14 article, the country's general prosecutor has responded to domestic campaigning and has stated that there is no law preventing homosexuals from donating blood. As a result of this inquiry, the general prosecutor has called on the Ministry of Health to rescind its guidance from 2001 that disallows the homosexual community from donating because of the belief that it was a group of high-risk individuals.

An audit of more than 8,000 transfusion episodes that took place at 217 U.K. hospitals reveals that health care personnel do not check the identity of bedside transfusion patients in 6 percent of all cases. Although the practice of confirming patients' identities is the single most important way to make sure they are given the correct type of blood, the Royal College of Physicians and the National Blood Service found that the practice was not happening as often as necessary. Even when this practice is observed, identifying bracelets do not always contain the necessary information. The audit noted that in 9 percent of wristbands worn, key data were missing. Reportedly, the main reason for not reconfirming the transfusion recipient's identity was that the patient was "well known" by nurses and physicians. The audit also revealed that 34 percent of patients were not monitored during the first 30 minutes following the transfusion, when it is critical to observe vital signs for indications of adverse events.

The implications of the findings are that more staff training is needed, especially to review the hazards and correct procedures for blood transfusion. The National Blood Transfusion Committee and the National Patient Safety Agency have also considered initiatives to overcome these shortcomings, such as improved use of automated systems and barcode patient identification.

After a year of study utilizing a new machine designed to clean the prions causing Creutzfeldt-Jakob disease (CJD) from donated blood, the Irish Blood Transfusion Service has decided to discontinue the trials. According to a July 11 *Irish Examiner* article, the transfusion service purchased the filter system last year but has halted its use due to concerns that it was not working sufficiently and that CJD could still pass undetected through blood donations.

The Irish Blood Transfusion Service also recently released its annual report for 2005, in which it revealed that blood donations increased 1.4 percent last year in tandem with increased need.

People

The lieutenant governor of Arkansas died last week after two bone marrow transplants failed to cure his unclassified myeloproliferative disorder. According to a July 16 *Reuters* story, Win Rockefeller was in the middle of a gubernatorial campaign when he was diagnosed with the blood disorder last July. He received treatment in both Washington and Arkansas, but died July 16 at the age of 57. The public servant had hoped to follow in the footsteps of his late father, who served as the governor of Arkansas from 1966 to 1970. The former governor died of cancer at the age of 60.

Upcoming Events

July

26 **Benchmarking**
AABB Audioconference
Contact: AABB Education Department
Tel: +1.301.215.6842
Fax: +1.301.215.6895
Email: education@aabb.org
Online: www.aabb.org/Content/Meetings_and_Events/Audioconferences/audio_conf.htm

31 – August 2 **ASQ's 2006 Quality Institute for Healthcare Conference**
Houston, TX
Contact: ASQ
Tel: +1.414.298.8789 x7263
Email: fspano@asq.org
Online: www.asq.org

August

9 **Transfusion When Nothing is Compatible: Evaluating and Managing Risk**
AABB Audioconference
Contact: AABB Education Department
Tel: +1.301.215.6842
Fax: +1.301.215.6895
Email: education@aabb.org
Online: www.aabb.org/Content/Meetings_and_Events/Audioconferences/audio_conf.htm

医薬品 研究報告 調査報告書

識別番号・報告回数			報告日	第一報入手日	新医薬品等の区分	機構処理欄
				2006. 5. 22	該当なし	

一般的名称	(製造承認書に記載なし)	研究報告の公表状況	Robertson C, Booth SA, Beniac DR, Coulthart MB, Booth TF, McNicol A. Blood. 2006 May 15;107(10):3907-11.	公表国
販売名(企業名)	合成血「日赤」(日本赤十字社) 照射合成血「日赤」(日本赤十字社)			カナダ

研究報告の概要

○細胞プリオン蛋白は活性血小板のエキソソーム上に放出されている

細胞プリオン蛋白(PrP^C)は、未知の機能を持つGPIアンカー型蛋白である。血球成分を含め体内の多くの組織に見られ、このうちヒトでは血小板で最も多く検出される。ミスフォールドされたプロテアーゼ耐性型PrP^CであるPrP^{Sc}が、伝達性海綿状脳症(TSE)グループの致死性神経変性疾患の病因であると広く信じられてきた。TSEの病因については完全にはわかっていないものの、疾患の進行にはPrP^Cが必要であることが知られている。故にPrP^Cの生理学的機能を明らかにすることが重要である。血中のPrP^Cの位置を解明することは、PrP^Cの機能に関して有益な手がかりを提供することになるだろう。PrP^Cは、以前は静止血小板のα顆粒膜上に見られた。最近の研究から、血小板の活性化が血小板表面上のPrP^Cの一時的発現とそれに続く微小胞およびエキソソームへの放出につながることが明らかになった。血小板由来エキソソーム上のPrP^Cの存在は、血中のPrP^Cの運搬と細胞間伝播のメカニズムの可能性を示唆する。

使用上の注意記載状況・その他参考事項等

合成血「日赤」
照射合成血「日赤」

血液を介するウイルス、細菌、原虫等の感染
vCJD等の伝播のリスク

報告企業の意見	今後の対応
PrP^Cは、静止血小板のα顆粒膜上に見られたが、血小板の活性化が血小板表面上のPrP^Cの一時的発現とそれに続く微小胞およびエキソソームへの放出につながることが明らかになったとの報告である。	今後も引き続き、プリオン病に関する新たな知見及び情報の収集に努める。

Cellular prion protein is released on exosomes from activated platelets

Catherine Robertson, Stephanie A. Booth, Daniel R. Beniac, Michael B. Coulthart, Timothy F. Booth, and Archibald McNicol

Cellular prion protein (PrP^C) is a glycophosphatidylinositol (GPI)–anchored protein, of unknown function, found in a number of tissues throughout the body, including several blood components of which platelets constitute the largest reservoir in humans. It is widely believed that a misfolded, protease-resistant form of PrP^C, PrP^{Sc}, is responsible for the transmissible spongiform encephalopathy (TSE) group of fatal neurodegenerative diseases. Although the pathogenesis of TSEs is poorly understood, it is known that PrP^C must be present in order for the disease to progress; thus, it is important to determine the physiologic function of PrP^C. Resolving the location of PrP^C in blood will provide valuable clues as to its function. PrP^C was previously shown to be on the alpha granule membrane of resting platelets. In the current study platelet activation led to the transient expression of PrP^C on the platelet surface and its subsequent release on both microvesicles and exosomes. The presence of PrP^C on platelet-derived exosomes suggests a possible mechanism for PrP^C transport in blood and for cell-to-cell transmission. (Blood. 2006;107: 3907-3911)

Introduction

Cellular prion protein (PrP^C) is a membrane-bound, glycophosphatidylinositol (GPI)–anchored protein[1] found primarily in lipid rafts on the cell membrane of neuronal and non-neuronal cells, including tonsils, spleen, and of the secretory granules of epithelial cells in the stomach, as well as in cultured cell lines.[2,3] Although PrP^C has been shown to be present on the surface of a number of peripheral blood cells,[4] the relative levels on individual cell types have been contentious. Individual studies have reported that the majority of PrP^C is associated with both platelets[5,6] and red blood cells.[7] In the former case the surface expression of PrP^C is increased following stimulation, suggesting an additional internal membrane source of the protein,[8] recently shown to be alpha granule membranes.[9] Furthermore, platelet activation is associated with the accumulation of PrP^C in releasates,[10] and in platelet concentrates, stored for up to 10 days, there is an increase in initially the microsomal, then plasma levels of PrP^C.[11]

Transmissible spongiform encephalopathies (TSEs) are a family of neurodegenerative disorders, including Creutzfeldt-Jakob disease (CJD), Gerstmann-Straussler-Schienker syndrome, and fatal familial insomnia in humans; scrapie in sheep; and bovine spongiform encephalopathy (BSE) in cattle.[12,13] They are all characterized by the accumulation of a protease-resistant isomer (PrP^{Sc}) of PrP^C in the brain of affected individuals. It is generally considered that PrP^{Sc} acts as a template inducing the same structural changes within other normally folded PrP^C molecules on contact, thus propagating the misfolded state of the protein.[14]

The CNS is the site at which TSE pathology is apparent in prion infections; however, the agent must first replicate and be transported to the CNS after peripheral infection. The spread of PrP^{Sc} has been tracked from the gastrointestinal tract and the spleen to the CNS.[15] The lymphoreticular system (LRS) is believed to be an important site of prion replication, and an accumulation of PrP^{Sc} is apparent in spleen and lymph nodes after peripheral infection. Indeed, neuroinvasion is delayed without a functional LRS.[16]

A new variant of CJD (vCJD) identified in humans in the United Kingdom is almost certainly the result of infection with the BSE agent.[17] Patients infected with vCJD, in contrast to those with classic CJD, have been shown to have widespread deposition of PrP^{Sc} in the LRS.[18,19] Immune cells, in particular B cells and follicular dendritic cells, have been identified as harboring infectious PrP^{Sc} in the LRS. However PrP^{Sc}, the standard biochemical marker used for diagnosis of TSEs, cannot be detected by current technology in circulating lymphocytes or whole blood. Bioassays are a more sensitive assay for infectivity, and a number of studies have demonstrated that the infectious agent is present in blood and blood components, buffy coats, plasma, and platelets in animal models.[20-23] The removal of all white cells by standard leukoreduction reduced infectivity by only 42%, suggesting that other blood components carry PrP^{Sc}.[24] There is therefore a significant concern that blood transfusions may represent a portal for the transmission of TSEs. Indeed since 2004, 3 apparent cases of transmission of vCJD by transfusion have been reported in the United Kingdom.[25,26]

To understand the transmission of the disease by PrP^{Sc}, it is important to determine the physiologic behavior and function of normal PrP^C in blood cells and plasma. In the current study the cellular localization, and stimulus-induced redistribution, of PrP^C in platelets has been examined.

From the Department of Oral Biology, University of Manitoba, Winnipeg, MB, Canada; the Department of Medical Microbiology, University of Manitoba, Winnipeg, MB, Canada; the Department of Pharmacology and Therapeutics, University of Manitoba, Winnipeg, MB, Canada; the Division of Host Genetics and Prion Diseases, Canadian Science Centre for Human and Animal Health, Winnipeg, MB, Canada; and the Division of Viral Diseases, Canadian Science Centre for Human and Animal Health, Winnipeg, MB, Canada.

Submitted February 28, 2005; accepted January 13, 2006. Prepublished online as *Blood* First Edition Paper, January 24, 2006; DOI 10.1182/blood-2005-02-0802.

Reprints: A. McNicol, Department of Oral Biology, 780 Bannatyne Ave, Winnipeg, MB, Canada, R3E 0W2; e-mail: mcnicol@ms.umanitoba.ca.

The publication costs of this article were defrayed in part by page charge payment. Therefore, and solely to indicate this fact, this article is hereby marked "advertisement" in accordance with 18 U.S.C. section 1734.

Materials and methods

The protocols of this study were approved by the Human Research Ethics Board of the University of Manitoba.

Antibodies and reagents

Monoclonal antibody (Ab) 308, raised against amino acids 106 to 126 of human PrP^C, was purchased from Cayman Chemical, Ann Arbor, MI, and the polyclonal Ab FL253, raised against the full-length PrP^C, was purchased from Santa Cruz Biotechnology, Santa Cruz, CA. Anti-CD62P Ab (D541) was a generous gift from Dr Sara Israels, Manitoba Institute of Cell Biology, Winnipeg, MB.

Polyclonal Ab to human fibrinogen was purchased from Calbiochem-Novabiochem, San Diego, CA. Horseradish peroxidase– and FITC-conjugated Abs were purchased from DakoCytomation, Mississauga, ON. Secondary Abs conjugated to gold, along with bovine thrombin, protease inhibitors, and all other reagents were purchased from Sigma-Aldrich Canada, Oakville, ON, and were of the highest grade available.

Preparation of washed platelets

Blood was collected following informed consent, by venipuncture of human volunteers who had denied taking medication known to interfere with platelet function within the previous 2 weeks. The blood was drawn into syringes containing acid citrate dextrose anticoagulant (ACD; 3.8 mM citric acid,.7.5 mM trisodium citrate, 125 mM dextrose; 1.8 mL anticoagulant/8.1 mL whole blood). Washed platelets were obtained as previously described.[27,28]

Flow cytometry

Plasma-free platelet suspensions were prepared and incubated with agonist, or saline control, for the times indicated. The samples were fixed by the addition of an equal amount of 4% paraformaldehyde, then incubated with anti-PrP^C Ab 308 diluted in PBS/0.1% BSA. Following washing 3 times in PBS/0.1% BSA, the samples were incubated in a FITC-conjugated secondary Ab. The samples were finally washed 3 times in PBS and resuspended in PBS. Flow cytometry was carried out on a Becton Dickinson (Mississauga, ON, Canada) FacsCalibur flow cytometer with forward and side scatter set to a logarithmic scale.

An area was drawn around the unstimulated platelet sample according to the forward and side scatter properties and labeled platelet region (P). A second region was drawn to gate on smaller particles and labeled microvesicle (MVS) region.[29] Fluorescence backgating was used to determine the number of PrP^C-positive events in each region.

Microvesicle and exosome preparation

Microvesicles and exosomes were prepared according to the method of Heijnen et al.[29] Briefly, plasma-free platelet suspensions were prepared and incubated with agonist or saline control for the times indicated, and the reaction was stopped by the addition of an equal amount of ice-cold ACD. Samples were centrifuged (Beckman GH-3 swing bucket rotor [Beckman Coulter Canada, Mississauga, ON, Canada]; 800*g*; 15 minutes) to obtain the platelet pellet fraction (P). The supernatant was further centrifuged (Beckman F3602 fixed angle rotor [Beckman Coulter Canada, Mississauga, ON, Canada]; 10 000*g*; 30 minutes) to obtain the microvesicle fraction (MV), and the supernatant from the MV fraction was centrifuged (Beckman MLS-50 swing bucket rotor; 100 000*g*; 60 minutes) to obtain the exosome pellet (EX). All pellets were resuspended in PBS.

To ensure exosome isolation, the supernatants from MV preparations were mixed with 2 M sucrose, and a 0.8 to 0.25 M sucrose gradient was layered on top. The gradient was centrifuged (Beckman MLS-50 swing bucket rotor; 65 000*g*; 16 hours) and 500-μL fractions were collected. The fractions were diluted in 4.5 mL PBS and centrifuged (100 000*g*; 60 minutes; 4°C), and the resultant pellets were resuspended in PBS.

In some studies, fractions from the sucrose gradient were vacuum-transferred onto a nitrocellulose membrane using a Schleicher and Schuell (Dassel, Germany) slot blot apparatus and immunoblotted with anti-PrP^C Ab. Briefly, following blocking in 5% nonfat milk, the membrane was incubated in Ab 308, washed in TBS with 0.01% Tween 20 (TBS-T) followed by incubation in a horseradish peroxidase–conjugated secondary Ab. After washing in TBS-T 6 times, the membrane was incubated in Super Signal West Pico chemiluminescence substrate (Pierce Biotechnology, Rockford, IL), and protein bands were visualized on a Bio-Rad Fluoro S Max imaging system. Densitometry was performed using Quantity One software (Bio-Rad, Mississauga, ON).

Electron microscopy and immunocytochemistry

Exosomes for double immunolabeling studies were prepared from the sucrose gradient fractions. Briefly, the exosomes were resuspended in PBS and adsorbed onto 300 mesh carbon-coated formvar nickel grids for 10 minutes. Excess PBS was blotted with filter paper, and the grids were fixed with 2% paraformaldehyde in PBS for 10 minutes. After fixation, the grids were incubated in PBS with 1% BSA for 15 minutes, incubated with the anti-PrP^C Ab for 30 minutes, washed 3 times in PBS with 0.1% BSA, and incubated in a secondary Ab conjugated to 5 nm gold for 30 minutes. The grids were rewashed 3 times in PBS, followed by 3 changes of PBS with 1% BSA, incubated in the anti-CD62 Ab for 30 minutes, washed (3 × 5 minute) with PBS with 0.1% BSA, and incubated in a secondary Ab conjugated to 10 nm gold for 30 minutes. Finally, the grids were washed (3 × 5 minutes) with PBS, followed by deionized water (3 × 5 minutes), embedded in uranyl acetate methyl cellulose. All specimens were examined in a Tecnai 20 transmission electron microscope (FEI Systems, Toronto, ON, Canada) operating at 200 kV at magnifications ranging from 25 500 × to 135 000 ×. Digital images (1024 × 1024-pixel) were acquired from the TEM via an AMT Advantage XR-12 TEM camera (AMT, Danvers, MA). Digital images were arranged in final figure format using the Canvas software package (ACD Systems of America, Miami, FL).

Frozen sections of platelets were prepared according to a method by Tokuyasu,[30] as previously described.[31] Cryo sections were cut with an Ultracut UCT ultramicrotome (Leica Microsystems Canada, Richmondhill, ON), equipped with an electron microscope (EM) FCS-sectioning chamber. Immunolabeling was carried out as described for exosome preparations with gold-conjugated protein G replacing the secondary Ab. Following gold labeling, the sections were stained and embedded in a uranyl acetate/methyl cellulose mixture.[30,31]

Results

PrP^C is present on platelet alpha granule, but not dense granule, membranes

Frozen sections of quiescent human platelets showed the characteristic platelet intracellular architecture (Figure 1A). Immunoelectron microscopy studies of these platelets using an anti-PrP^C Ab, FL253, followed by gold-conjugated Protein G, confirmed the association of PrP^C with intracellular granules (Figure 1B-C) and the membranes of the open canalicular system (Figure 1C). A polyclonal Ab to fibrinogen was used to identify alpha granules (Figure 1D), and double staining of these sections using Abs to PrP^C and fibrinogen was consistent with both proteins localizing to the same organelle (Figure 1E-F).

PrP^C translocates to and is released from the platelet surface following activation

Lysates from thrombin-stimulated platelets (1 U/mL; 0-120 seconds) were immunoblotted with anti-PrP^C Ab 308. There was a decrease in PrP^C levels associated with the platelet pellet over time (Figure 2A). Although there was significant donor variability in the time course of the loss of PrP^C from the pellet, densitometric analysis indicated that by 120 seconds of stimulation the intensity

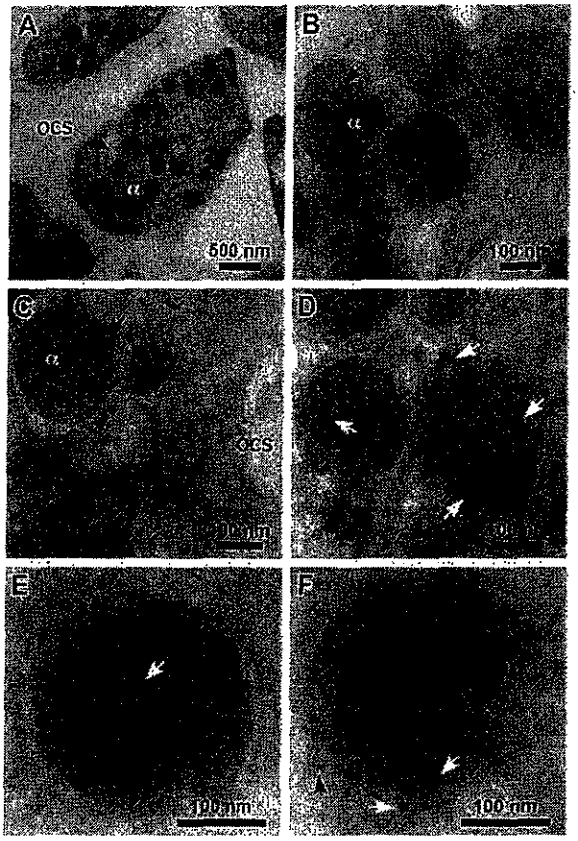


Figure 1. Immunoelectron microscopy of resting platelets. Frozen sections of resting platelets were incubated with Abs to PrP^C (FL253) and fibrinogen, followed by incubation in protein G conjugated to 5 or 10 nm gold. Normal resting platelets showing alpha granule (α) and open canalicular system (OCS) (A). PrP^C (10 nm gold, arrows) is seen in alpha granule and the open canalicular system (B-C). Alpha granules are identified by the presence of fibrinogen (5 nm gold, arrows; D). Double labeling with Abs to PrP^C (5 nm gold, black arrows) and fibrinogen (10 nm gold, white arrows) localized both proteins to the same granules (E-F).

of the PrP^C band from the platelet pellet had diminished to about 50% of control (Figure 2B). This decrease in platelet-associated PrP^C from activated platelets was accompanied by a corresponding accumulation of PrP^C in the releasate (Figure 2A-B).

PrP^C is present on platelet microvesicles

Flow cytometry, using anti-PrP^C Ab 308, demonstrated the presence of PrP^C on the surface of unstimulated platelets (Figure 3). Following thrombin stimulation (1 U/mL) there was a transient increase in the expression of PrP^C on the platelet surface, followed by its release into the supernatant. There was a corresponding shedding of microvesicles from the platelet surface in response to thrombin; however, the level of PrP^C on the surface of the microvesicles was low (< 10% of total released PrP^C) and remained constant with time (Figure 3). Platelet activation with A23187 (20 μM) was accompanied by increased levels of microvesicle production when compared with thrombin; however, the level of PrP^C associated with the microvesicles remained low (data not shown).

Immunogold labeling demonstrated the presence of PrP^C on the surface of unstimulated platelets (Figure 4A). Following activation with thrombin, PrP^C was observed around the periphery of the platelet and at the tips of pseudopods (Figure 4B-C). In addition, PrP^C was associated with small (< 100 nm) membranous vesicles released from the platelets (Figure 4D-E).

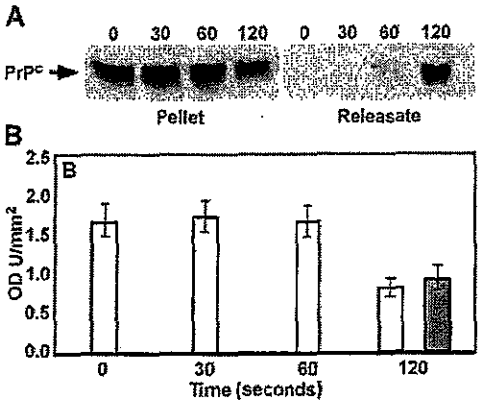


Figure 2. Immunoblotting of pellets and releasates in activated platelets. Platelets were incubated with 1 U/mL thrombin for 0, 30, 60, or 120 seconds. Following termination, platelet pellet and releasates were prepared, separated by sodium dodecyl sulfate–polyacrylamide gel electrophoresis, and immunoblotted with anti-PrP^C Ab 308 (A), and densitometry was performed (B) on pellet (□) and releasates (▨) (n = 3). Error bars indicate standard error of the mean.

PrP^C is present on platelet exosomes

Previous studies have shown that, in addition to microvesicles, thrombin stimulates the release of exosomes from platelets.[29] Given the relatively low levels of PrP^C on the surface of released microvesicles (Figure 3) and its presence on the surface of smaller membrane fractions (Figure 4D-E), the possible association of PrP^C with exosomes was examined.

Exosomes were prepared by differential centrifugation, and separation through a sucrose gradient, of the releasate of thrombin-stimulated platelets. Immunoblotting using anti-PrP^C Ab 308 was consistent with the presence of PrP^C in these exosome fractions (Figure 5A).

Immunoelectron microscopy of fractions 3 and 4 using anti-PrP^C Abs (308 or FL253) demonstrated the presence of PrP^C on vesicles ranging from 40 to 100 nm (Figure 5B), the size being consistent with previous reports of platelet-derived exosomes.[29] Double-labeling these fractions with anti-CD62 Ab D541 confirmed that these exosomes were derived from alpha granules (Figure 5C).

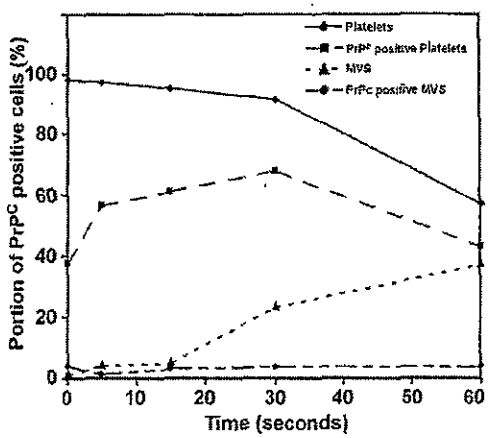


Figure 3. Flow cytometry of activated platelets. Platelets were incubated with 1 U/mL thrombin for 0, 5, 15, 30, or 60 seconds and labeled with anti-PrP^C Ab 308. Flow cytometry was used to distinguish platelets from microvesicles on the basis of their forward-scatter (FSC-H) and side-scatter (SSC-H) profiles (platelets, solid line ◆, microvesicles, broken line ▲). Fluorescence backgating determined the percentage of PrP^C-positive cells in each region. (Percentage of platelet region positive for PrP^C, ■; percentage of microvesicle region positive for PrP^C, ●).

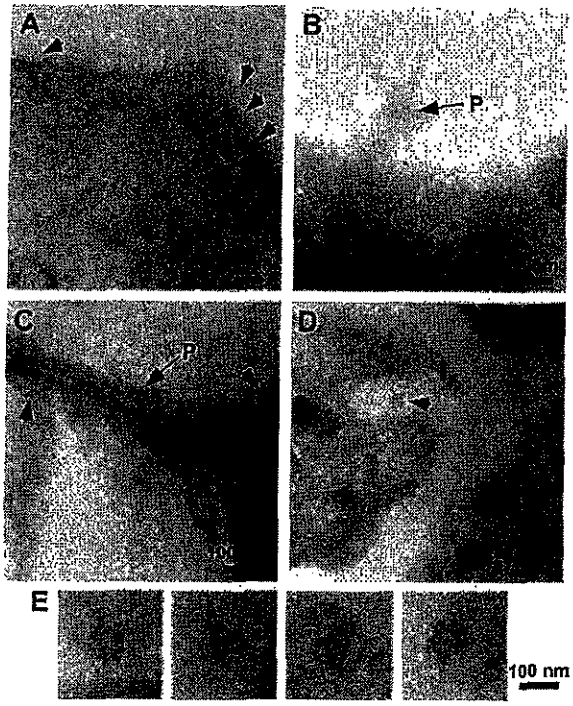


Figure 4. Immunogold labeling of resting and activated platelets. Resting and activated platelets were incubated with Abs to PrP^C (308 or FL253), followed by protein G or secondary antibodies conjugated to 10 nm gold (arrowheads). In resting platelets preincubated with Ab 308 before embedding, PrP^C is seen around the periphery of the cell (A). In activated platelets PrP^C is found at the periphery of the cell and is associated with pseudopods (P, arrows) (B, whole mount; C, frozen section). In frozen sections of activated platelets labeled with Ab FL253, PrP^C was also localized to released exosomes (seen between cells, D; and at higher magnification, E).

Discussion

The current study localizes PrP^C to platelet alpha granule, but not dense granule, membranes, confirming a recent study by Starke et al.[9] Thus, PrP^C is present with proteins such as the αIIb/β3 integrin, CD62 (P-selectin), CD36, and the GPIb/V/IX complex[32-35] inherent in the alpha granule membrane, and, in common with these other proteins, there is an activation-mediated increase in expression of PrP^C on the external platelet surface. The function of PrP^C in platelets is unknown; preincubation with anti-PrP^C Abs has a limited effect on platelet adhesion to a variety of matrices but no effect on agonist-induced aggregation (Robertson et al, unpublished); therefore, it is unlikely that PrP^C plays a significant role in either of these platelet functions. In contrast to the expression of other activation-associated proteins, the thrombin-induced expression of PrP^C on the platelet surface was transient and was followed by its release. Previous studies have shown that PrP^C is present in platelet releasates[10]; however, the current study demonstrates that the released PrP^C is associated with membranes, initially in small quantities on microvesicles and subsequently in higher levels on exosomes.

Exosomes are small (40-100 nm), membrane-bounded vesicles which are released from a variety of cells following exocytosis[36] and are present in human plasma.[37] Denzer et al[38] reviewed a large number of proteins and lipids that are associated with exosomes, which include members of the tetraspanin protein family, the immunoglobulin supergene family, as well as GPI-anchored proteins and cytosolic proteins. Exosomes have been implicated in cell-to-cell communication mechanisms by transferal of proteins directly from the exosomes to target cells, in a manner similar to the movement of GPI-anchored proteins from the plasma membrane of red blood cells to endothelial cells.[39,40] Furthermore, exosomes have been implicated in the activation of the immune system, including the stimulation of T lymphocytes and a potential interaction with follicular dendritic cells.[38] Reticulocyte-derived exosomes may participate in complement regulation.[41] Interestingly, Whiteside[42] has recently proposed that exosomes play a role in the evasion of tumor cells from the immune system.

Studies in platelets have shown the release of alpha granule membrane–derived exosomes following exocytosis.[29] Therefore, the presence of PrP^C on exosomes is entirely consistent with the alpha granule membrane source of these vesicles. The function of platelet-derived exosomes is unknown, although the low binding of factor X, prothrombin, and annexin V to their surface suggests that they do not have the same procoagulant activity as platelet-derived microvesicles.[29] The expression of CD62 on the surface of platelet-derived exosomes points to a possible role in adhesion, or cell-to-cell transfer of adhesive properties, because CD62 is known to mediate adhesion between leukocytes and endothelial cells.[43]

The presence of prion protein on exosomes has recently been highlighted by Fevrier et al,[44] who reported the presence of infectious PrP^{Sc} in exosomes derived from cultured epithelial and neuroglial cell lines after infection with scrapie. They subsequently proposed that exosomes may provide a vehicle for transport of PrP^{Sc} from cell to cell, thus providing a mechanism for transmission of infectious proteins in the body.[45,46] The current finding that PrP^C is present on platelet-derived exosomes strengthens the hypothesis that exosome release is a general mechanism for transport of proteins and inferentially pathogen transmission,

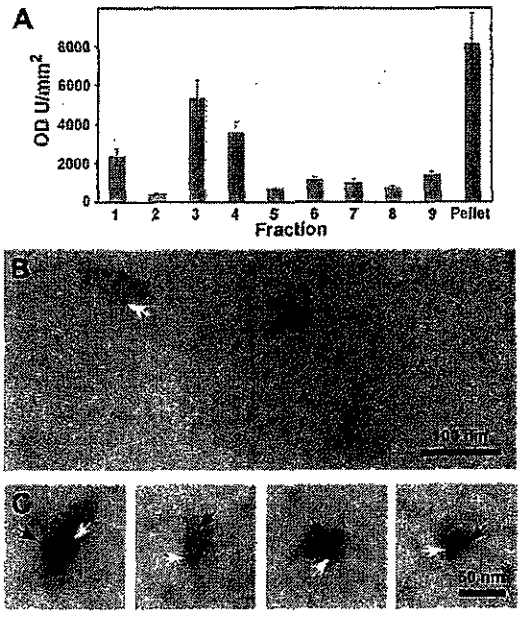


Figure 5. Immunoblotting and immunoelectron microscopy of isolated exosomes. Platelets were incubated with 1 U/mL thrombin for 120 seconds. Following termination, platelets were removed by centrifugation at 800*g*. Further centrifugation of the supernatant removed the microvesicles. Exosomes were isolated by differential centrifugation through a sucrose gradient. Fractions were collected from the top, and immunoblotting was carried out in each fraction using anti-PrP^C Ab 308. The blots were subjected to densitometry and are expressed as mean plus or minus standard error of the mean; n = 3 (A). Fractions 3 and 4 from the sucrose gradient were adsorbed onto formvar-coated grids and double labeled with anti-PrP^C Ab 308 followed by an anti-CD62 Ab (D541). The respective secondary Abs were conjugated to 5 nm (anti-PrP^C; black arrows) and 10 nm (anti-CD62; white arrows) gold (B-C).

including prions, between cells. Platelets contain a large proportion of circulating PrP^{C}[5,6]; therefore, platelet-derived exosomes could potentially act as an important source of protein for prion replication. In addition, the transferral of exosomes containing PrP^{C} to cell types in which it is normally absent may confer susceptibility to infection with prions. To date, this has not been addressed.

Although there is no biochemical evidence for the presence of PrP^{Sc} on platelets, a recent study by Cervenakova et al[23] identified prion infectivity in the platelet and plasma fractions of murine blood from mice infected with mouse-adapted vCJD. The present finding that PrP^{C} is released on exosomes from activated platelets therefore raises the possibility that PrP^{Sc} is similarly released from platelets. Although this has not been addressed in the current study, it is clearly plausible that the generation of PrP^{Sc}-containing platelet exosomes during preparation of blood products could account for the transmission of variant CJD by blood transfusion. Leukoreduction of plasma, a process which would not remove exosomes, reduced infectivity by only 42%[24] and, when taken in concert with the current study, suggests that further investigation into the possible role of platelet-derived exosomes as vehicles for prion transmission is clearly warranted.

Acknowledgments

We thank Ms Debra Godal for technical assistance and Dr Sara Israels for providing the monoclonal Ab D541 and for helpful discussions and critical reading of this manuscript.

References

1. Stahl N, Borchelt DR, Hsiao K, Prusiner SB. Scrapie prion protein contains a phosphatidylinositol glycolipid. Cell. 1987;51:229-240.
2. Vey M, Pilkuhn S, Wilte H, et al. Subcellular colocalization of the cellular and scrapie prion proteins in caveolae-like membranous domains. Proc Natl Acad Sci U S A. 1996;93:14945-14949.
3. Fournier JG, Escaig-Haye F, Billette de Villemeur T, et al. Distribution and submicroscopic immunogold localization of cellular prion protein (PrPc) in extracerebral tissues. Cell Tissue Res. 1998;292: 77-84.
4. Vostal JG, Holada K, Simak J. Expression of cellular prion protein on blood cells: potential functions in cell physiology and pathophysiology of transmissible spongiform encephalopathy diseases. Transfus Med Rev. 2001;15:268-281.
5. Barclay GR, Hope J, Birkett CR, Turner ML. Distribution of cell-associated prion protein in normal adult blood determined by flow cytometry. Br J Haematol. 1999;107:804-814.
6. MacGregor I, Drummond O, Turner M, Barclay R, Prowse C. Distribution of normal prion protein in blood. Transfus Sci. 2000;22:51.
7. Holada K, Vostal JG. Different levels of prion protein (PrPc) expression on hamster, mouse and human blood cells. Br J Haematol. 2000;110:472-480.
8. Holada K, Mondoro TH, Muller J, Vostal JG. Increased expression of phosphatidylinositol-specific phospholipase C resistant prion proteins on the surface of activated platelets. Br J Haematol. 1998;103:276-282.
9. Starke R, Harrison P, Mackie I, et al. The expression of prion protein (PrP(C)) in the megakaryocyte lineage. J Thromb Haemost. 2005;3:1266-1273.
10. Perini F, Vidal R, Ghetti B, et al. PrP27–30 is a normal soluble prion protein fragment released by human platelets. Biochem Biophys Res Commun. 1996;223:572-577.
11. Bessos H, Drummond O, Prowse C, Turner M, MacGregor I. The release of prion protein from platelets during storage of apheresis platelets. Transfusion. 2001;41:61-66.
12. Knight RS, Will RG. Prion diseases. J Neurol Neurosurg Psychiatry. 2004;75(Suppl 1):i36-i42.
13. Palmer MS, Collinge J. Human prion diseases. Curr Opin Neurol Neurosurg. 1992;5:895-901.
14. Prusiner SB. Prions. Proc Natl Acad Sci U S A. 1998;95:13363-13383.
15. Weissmann C, Enari M, Klohn PC, Rossi D, Flechsig E. Transmission of prions. Proc Natl Acad Sci U S A. 2002;99(Suppl 4):16378-16383.
16. Brown KL, Stewart K, Ritchie DL, et al. Scrapie replication in lymphoid tissues depends on prion protein-expressing follicular dendritic cells. Nat Med. 1999;5:1308-1312.
17. Bruce ME, Will RG, Ironside JW, et al. Transmissions to mice indicate that 'new variant' CJD is caused by the BSE agent. Nature. 1997;389:498-501.
18. Klein MA, Frigg R, Flechsig E, et al. A crucial role for B cells in neuroinvasive scrapie. Nature. 1997; 390:687-690.
19. Hilton DA, Sutak J, Smith ME, et al. Specificity of lymphoreticular accumulation of prion protein for variant Creutzfeldt-Jakob disease. J Clin Pathol. 2004;57:300-302.
20. Brown P, Cervenakova L, McShane LM, et al. Further studies of blood infectivity in an experimental model of transmissible spongiform encephalopathy, with an explanation of why blood components do not transmit Creutzfeldt-Jakob disease in humans. Transfusion. 1999;39:1169-1178.
21. Hunter N, Foster J, Chong A, et al. Transmission of prion diseases by blood transfusion. J Gen Virol. 2002;83:2897-2905.
22. Herzog C, Sales N, Etchegaray N, et al. Tissue distribution of bovine spongiform encephalopathy agent in primates after intravenous or oral infection. Lancet. 2004;363:422-428.
23. Cervenakova L, Yakovleva O, McKenzie C, et al. Similar levels of infectivity in the blood of mice infected with human-derived vCJD and GSS strains of transmissible spongiform encephalopathy. Transfusion. 2003;43:1687-1694.
24. Gregori L, McCombie N, Palmer D, et al. Effectiveness of leucoreduction for removal of infectivity of transmissible spongiform encephalopathies from blood. Lancet. 2004;364:529-531.
25. Llewelyn CA, Hewitt PE, Knight RS, et al. Possible transmission of variant Creutzfeldt-Jakob disease by blood transfusion. Lancet. 2004;363: 417-421.
26. Peden AH, Head MW, Ritchie DL, Bell JE, Ironside JW. Preclinical vCJD after blood transfusion in a PRNP codon 129 heterozygous patient. Lancet. 2004;364:527-529.
27. Lockhart LK, Pampolina C, Nickolaychuk BR, McNicol A. Evidence for a role for phospholipase C, but not phospholipase A2, in platelet activation in response to low concentrations of collagen. Thromb Haemost. 2001;85:882-889.
28. McNicol A, Jackson EC. Inhibition of the MEK/ERK pathway has no effect on agonist-induced aggregation of human platelets. Biochem Pharmacol. 2003;65:1243-1250.
29. Heijnen HF, Schiel AE, Fijnheer R, Geuze HJ, Sixma JJ. Activated platelets release two types of membrane vesicles: microvesicles by surface shedding and exosomes derived from exocytosis of multivesicular bodies and alpha-granules. Blood. 1999;94:3791-3799.
30. Tokuyasu KT. Immunochemistry on ultrathin frozen sections. Histochem J. 1980;12:381-403.
31. Israels SJ, McMillan EM, Robertson C, Singhory S, McNicol A. The lysosomal granule membrane protein, LAMP-2, is also present in platelet dense granule membranes. Thromb Haemost. 1996;75: 623-629.
32. Berger G, Caen JP, Berndt MC, Cramer EM. Ultrastructural demonstration of CD36 in the alpha-granule membrane of human platelets and megakaryocytes. Blood. 1993;82:3034-3044.
33. Berger G, Masse JM, Cramer EM. Alpha-granule membrane mirrors the platelet plasma membrane and contains the glycoproteins Ib, IX, and V. Blood. 1996;87:1385-1395.
34. Berger G, Hartwell DW, Wagner DD. P-Selectin and platelet clearance. Blood. 1998;92:4446-4452.
35. Michelson AD. Thrombin-induced down-regulation of the platelet membrane glycoprotein Ib-IX complex. Semin Thromb Hemost. 1992;18:18-27.
36. Thery C, Zitvogel L, Amigorena S. Exosomes: composition, biogenesis and function. Nat Rev Immunol. 2002;2:569-579.
37. Caby MP, Lankar D, Vincendeau-Scherrer C, Raposo G, Bonnerot C. Exosomal-like vesicles are present in human blood plasma. Int Immunol. 2005;17:879-887.
38. Denzer K, Kleijmeer MJ, Heijnen HF, Stoorvogel W, Geuze HJ. Exosome: from internal vesicle of the multivesicular body to intercellular signaling device. J Cell Sci. 2000;113(Pt 19):3365-3374.
39. Kooyman DL, Byrne GW, McClellan S, et al. In vivo transfer of GPI-linked complement restriction factors from erythrocytes to the endothelium. Science. 1995;269:89-92.
40. McCurry KR, Kooyman DL, Alvarado CG, et al. Human complement regulatory proteins protect swine-to-primate cardiac xenografts from humoral injury. Nat Med. 1995;1:423-427.
41. Kinoshita T, Medof ME, Hong K, Nussenzweig V. Membrane-bound C4b interacts endogenously with complement receptor CR1 of human red cells. J Exp Med. 1986;164:1377-1388.
42. Whiteside TL. Tumour-derived exosomes or microvesicles: another mechanism of tumour escape from the host immune system? Br J Cancer. 2005;92:209-211.
43. McEver RP. Adhesive interactions of leukocytes, platelets, and the vessel wall during hemostasis and inflammation. Thromb Haemost. 2001;86: 746-756.
44. Fevrier B, Vilette D, Archer F, et al. Cells release prions in association with exosomes. Proc Natl Acad Sci U S A. 2004;101:9683-9688.
45. Fevrier B, Vilette D, Laude H, Raposo G. Exosomes: a bubble ride for prions? Traffic. 2005;6: 10-17.
46. Porto-Carreiro I, Fevrier B, Paquet S, Vilette D, Raposo G. Prions and exosomes: from PrPc trafficking to PrPsc propagation. Blood Cells Mol Dis. 2005;35:143-148.

別紙様式第2-1

No. 14

医薬品 研究報告 調査報告書

識別番号・報告回数			報告日	第一報入手日	新医薬品等の区分	機構処理欄
				2006. 5. 22	該当なし	

一般的名称	(製造承認書に記載なし)	研究報告の公表状況	Ironside JW, Bishop MT, Connolly K, Hegazy D, Lowrie S, Le Grice M, Ritchie DL, McCardle LM, Hilton DA. BMJ. 2006 May 20;332(7551):1186-8.	公表国
販売名(企業名)	合成血「日赤」(日本赤十字社) 照射合成血「日赤」(日本赤十字社)			英国

研究報告の概要

○変異型クロイツフェルトヤコブ病(vCJD):有病率の後方視的研究から得られた陽性虫垂組織のプリオン蛋白の遺伝子型解析
目的:疾患関連プリオン蛋白陽性を示した虫垂組織から抽出したDNAのプリオン蛋白遺伝子(PRNP)コドン129の解析。
デザイン:英国における、変異型クロイツフェルトヤコブ病の連結不可能匿名化した後方視的有病率試験で判明した陽性例の再解析。
試験サンプル: 疾患関連プリオン蛋白の検査を実施した虫垂及び扁桃検体12,674件のうちの陽性例3検体。これらの検体を採取した患者の手術時(1996-1999年)の年齢は20-29歳であった。
実施場所:イングランド及びスコットランドの2箇所の第三次センターの病理学部門。
結果:3件のうち2件の検体について、適切なDNAが採取できたが、いずれもPRNPコドン129の遺伝子型はバリンのホモ接合体であった。
結論:PRNP中のコドン129がバリンのホモ接合体であるサブグループがvCJD感染に対する感受性があることが初めて示された。これまでvCJDの検査を受けた症例は、すべてメチオニンのホモ接合体サブグループであり、医原性vCJDと推定される1例のみがメチオニン/バリンのヘテロ接合だった。PRNPコドン129がバリンのホモ接合体であるvCJD患者の潜伏期間はより長期である可能性があり、輸血あるいは無症候の期間に患者に使用した外科用器具の汚染を介して、水平感染を起こす可能性がある。

使用上の注意記載状況・その他参考事項等

合成血「日赤」
照射合成血「日赤」

血液を介するウイルス、細菌、原虫等の感染
vCJD等の伝播のリスク

報告企業の意見	今後の対応
vCJD有病率の後方視的研究から、PRNPのコドン129がのホモ接合体であるサブグループがvCJD感染に対する感受性があることが初めて示されたとの報告である。	日本赤十字社は、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)を確認し、英国を含む欧州36ヶ国に一定期間滞在したドナーを無期限に献血延期としている。また、英国滞在歴を有するvCJD患者が国内で発生したことから、平成17年6月1日より1980年～1996年に1日以上の英国滞在歴のある方からの献血を制限している。さらに、感染リスク低減の目的から、血液製剤の保存前白血球除去の導入を進めている。今後も、CJD等プリオン病に関する内外の新たな知見及び情報の収集に努める。

22

Variant Creutzfeldt-Jakob disease: prion protein genotype analysis of positive appendix tissue samples from a retrospective prevalence study

James W Ironside, Matthew T Bishop, Kelly Connolly, Doha Hegazy, Suzanne Lowrie, Margaret Le Grice, Diane L Ritchie, Linda M McCardle and David A Hilton

BMJ 2006;332;1186-1188; originally published online 10 Apr 2006; doi:10.1136/bmj.38804.511644.55

What is already known on this topic

The evidence base for prescribing drugs to children lacks sufficient pharmacokinetic and pharmacodynamic data

Adult doses are often extrapolated to children without taking account of potential differences in drug handling with age or dose requirements for effectiveness

Licensing data for paediatric dosing are often sparse, and subsequent studies may result in important changes to recommended doses

What this study adds

HIV infected UK and Irish children have been underdosed with antiretrovirals in the past nine years

Poor pharmacokinetic data at licensing results in incorrect drug dosing until important pharmacokinetic results emerge after licensing and inform revision of dosage recommendations

Guidelines stating alternative dosage strategies (by weight or surface area) for the same drug lead to different and inconsistent doses

Inadequate dosing also arises through failure to adjust for ongoing growth

the United States have recently committed to promoting research specific to children's medicines while protecting children as participants in clinical trials. The UK Department of Health has launched the Medicines for Children Research Network (www.liv.ac.uk/mcrn), which aims to develop closer links between the drugs industry, regulators, families, and paediatricians, links that will be needed to meet the challenges of developing and manufacturing appropriate paediatric drugs (www.hivforum.org).

The Collaborative HIV Paediatric Study (CHIPS) is a collaboration between the Medical Research Council Clinical Trials Unit, UK, and the National Study of HIV in Pregnancy and Childhood (NSHPC) at the Institute of Child Health, London. Committees and participants are on bmj.com.

Contributors: See bmj.com

Funding: CHIPS is funded by the London HIV Consortium and in the past has received additional support from Bristol-Myers Squibb, Boehringer Ingelheim, GlaxoSmithKline, Roche, Abbott, and Gilead

Competing interests: None declared.

Ethical approval: UK multicentre research ethics committee and relevant local research ethic committees.

1 Kauffman RE. Clinical trials in children: problems and pitfalls. *Paediatr Drugs* 2000;2:411-8.
2 Caldwell PHY, Murphy SB, Butow ON, Craig JC. Clinical trials in children. *Lancet* 2004;364:803-11.
3 Turner S, Longworth A, Nunn AJ, Choonara I. Unlicensed and off label drug use in paediatric wards: prospective study. *BMJ* 1998;316:343-5.
4 Gibb DM, Duong T, Tookey PA, Sharland M, Tudor-Williams G, Novelli V, et al, on behalf of the National Study of HIV in Pregnancy and Childhood (NSHPC) and of the Collaborative HIV Paediatric Study (CHIPS). Decline in mortality, AIDS and hospital admissions in perinatally HIV-1 infected children in the UK and Ireland. *BMJ* 2003;327:1019-25.
5 Sharland M, Blanche S, Castelli-Gattinara G, Ramos JT, Gibb DM, PENTA Steering Committee. PENTA guidelines for the use of antiretroviral therapy. *HIV Medicine* 2004;5:61-86. www.pentatrials.org/guidelines.htm
6 Alexanian A, Gibbons S, Lyall EGH, Walters S, Tudor-Williams G. Higher nevirapine doses correlate with improved outcomes in a paediatric population. *12th Conference on Retroviruses and Opportunistic Infections.* Boston, Ma, February 2005 [Abstract PL11.2].
7 Jong GW, Eland IA, Sturkenboom MC, Van Den Anker JN, Stricker BH. Unlicensed and off label prescription of drugs to children: population based cohort study. *BMJ* 2002;324:1313-4.

(Accepted 6 March 2006)

Variant Creutzfeldt-Jakob disease: prion protein genotype analysis of positive appendix tissue samples from a retrospective prevalence study

James W Ironside, Matthew T Bishop, Kelly Connolly, Doha Hegazy, Suzanne Lowrie, Margaret Le Grice, Diane L Ritchie, Linda M McCardle, David A Hilton

Editorial by Wilson and Ricketts

Correspondence to: J W Ironside james.ironside@

continued over

BMJ 2006;332:1186–8

Abstract

Objective To perform prion protein gene (*PRNP*) codon 129 analysis in DNA extracted from appendix tissue samples that had tested positive for disease associated prion protein.

Design Reanalysis of positive cases identified in a retrospective anonymised unlinked prevalence study of variant Creutzfeldt-Jakob disease (vCJD) in the United Kingdom.

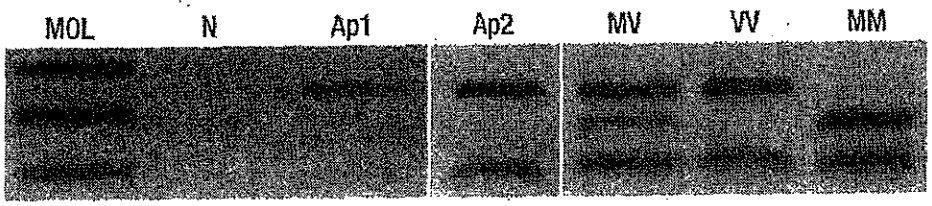
Study samples Three positive appendix tissue samples out of 12 674 samples of appendix and tonsil tested for disease associated prion protein. The patients from whom these samples were obtained were aged 20-29 years at the time of surgery, which took place in 1996-9.

Setting Pathology departments in two tertiary centres in England and Scotland.

Results Adequate DNA was available for analysis in two of the three specimens, both of which were homozygous for valine at codon 129 in the *PRNP*.

Conclusions This is the first indication that the valine homozygous subgroup at codon 129 in the *PRNP* is susceptible to vCJD infection. All tested clinical cases of vCJD have so far occurred in the methionine homozygous subgroup, and a single case of probable iatrogenic vCJD infection has been identified in one patient who was a methionine/valine heterozygote at

This article was posted on bmj.com on 10 April 2006: http://bmj.com/cgi/doi/10.1136/bmj.38804.511644.55



Restriction digest pattern for *PRNP* codon 129 genotype analysis in two paraffin section tissue samples (shown combined). The test sample results clearly show banding patterns equivalent to the VV genotype control (Mol=molecular weight ladder, N=PCR negative control, Ap1=appendix tissue from positive case 2, Ap2=appendix tissue from positive case 3, positive control samples from *PRNP* codon 129 MV, VV, and MM genotypes)

National Creutzfeldt-Jakob Disease Surveillance Unit, School of Molecular and Clinical Medicine, University of Edinburgh, Western General Hospital, Edinburgh EH4 2XU
James W Ironside *professor of clinical neuropathology*
Mathew T Bishop *geneticist*
Kelly Connolly *genetics technician*
Suzanne Lowrie *biomedical scientist*
Margaret Le Grice *biomedical scientist*
Diane L Ritchie *research assistant*
Linda McCardle *biomedical scientist*
Department of Histopathology, Derriford Hospital, Plymouth PL6 8DSH
Doha Hegazy *research technician*
David A Hilton *consultant neuropathologist*

this genetic locus. People infected with vCJD with a valine homozygous codon 129 *PRNP* genotype may have a prolonged incubation period, during which horizontal spread of the infection could occur either from blood donations or from contaminated surgical instruments used on these individuals during the asymptomatic phase of the illness.

Introduction

In a prevalence study for variant Creutzfeldt-Jakob disease (vCJD), we identified three appendixes that stained positively for disease associated prion protein (PrP). We looked at 12 674 specimens (11 109 appendices, 1565 tonsils) removed from 1995-2000. Most of the patients (83%) were aged 10-30 years at the time of operation.[1 2] This number of positive results is greater than would be predicted from the numbers of patients diagnosed with vCJD in the United Kingdom (161 to date). Furthermore, the annual incidence of new cases of vCJD has declined from a peak in 1999. As all patients with vCJD belong to the methionine homozygous subgroup, determined by the codon 129 polymorphism in the prion protein gene (*PRNP*),[2] one possible explanation for this apparent discrepancy could be a different *PRNP* genotype in the three positive cases (the prevalences of *PRNP* codon 129 genotypes in the general UK population are about 40% methionine homozygous, 10% valine homozygous, and 50% heterozygous). This possibility was supported by a slightly different pattern of immunoreactivity in the second and third positive appendix cases in comparison with clinical cases of vCJD.[2] We recently identified a case of asymptomatic vCJD infection that seemed to have been transmitted by red cell transfusion in a *PRNP* codon 129 heterozygote, demonstrating that the methionine homozygous genotype is not uniquely susceptible to vCJD infection.[3]

Methods

We analysed the *PRNP* codon 129 polymorphism in the three samples of appendix tissue embedded in paraffin that stained positively for disease associated prion protein in the prevalence study. In the first case, a transmission study is currently under way using material from the remaining unstained sections. This meant that only immunostained sections were available for genotype studies and the extracted DNA was not good enough for further analysis. In the two remaining cases, as there was not sufficient material available for both transmission studies and genotype studies, and in view of possible *PRNP* influences on the staining pattern of disease associated prion protein in these cases, we used the remaining material for DNA analysis. A single 6 µm unstained paraffin section was available from each case, and these were de-paraffinised and scraped into individual microcentrifuge tubes for DNA extraction with the Puregene DNA Purification Kit (Gentra Systems, USA). Pelleted DNA was rehydrated for one hour at 65°C and then used as a template for amplification by the polymerase chain reaction (PCR), along with positive and negative control samples. PCR primers used were specific for a 506 bp region of *PRNP* containing the polymorphic sequence for the codon 129 residue. PCR products were digested at 37°C with the restriction enzyme Nsp1 (New England Biolabs, UK), which specifically recognises changes at the *PRNP* codon 129 polymorphic DNA sequence. Digest products were analysed on 1.5% agarose gels with positive controls for the codon 129 variants (MV, VV, and MM).

Results

For both cases the genotype was confirmed as homozygous for the valine allele (VV) (figure). This method has been previously validated[4 5] and was controlled in our laboratory by studying the *PRNP* codon 129 genotype in both paraffin embedded sections and frozen tissues from 25 other cases.

Discussion

These results give the first indication that *PRNP* codon 129 valine homozygotes may be susceptible to vCJD infection. Though the immunohistochemical technique used in our earlier study seems to be specific for disease associated prion protein,[6] it is unlikely to be 100% sensitive, suggesting that the true prevalence of vCJD infection in the UK population may be even higher than earlier estimated (3/12 674).[2] Genetic studies of kuru, another orally transmitted human prion disease, found that *PRNP* codon 129 MV and VV genotypes were associated with longer incubation periods than the MM genotype.[7] As the ethical approval for our study placed restraints on the identification of individual cases, we are not able to state with certainty the age of the patients in the positive cases at the time of surgery. We can, however, state that they were aged 20-29 years at the time of surgery, which took place in 1996-9. No clinical cases of vCJD at any age have yet been identified in *PRNP* codon 129 valine homozygotes, indicating the need for continued surveillance of all cases of vCJD in the UK.

What is already known on this topic

A recent prevalence study of accumulation of prion protein (as a marker for vCJD infection) in appendix and tonsil specimens in the UK found 3/12 674 positive cases, which is more than expected from the current number of clinical cases of vCJD

What this study adds

Analysis of DNA from two of the three positive samples found them to be valine homozygotes at codon 129 in the prion protein gene, indicating that this genetic subgroup (which is a different subgroup from that in which all cases of vCJD have so far occurred) is susceptible to vCJD infection

Individuals with this genotype may have a prolonged incubation period with subclinical infection and could cause secondary spread of vCJD by blood transfusion or surgery

Though it is inadvisable to overinterpret the data from only three positive cases in this study, it is perhaps surprising (given the relative prevalences of *PRNP* codon 129 genotypes in the general population) that both the positive cases analysed here were valine homozygotes. Though this may represent a chance finding, we should consider the possibility of differences in the peripheral pathogenesis of vCJD that depend on the *PRNP* codon 129 genotype. The patient who developed asymptomatic vCJD infection after red blood cell transfusion was a codon 129 heterozygote in whom both tonsil and appendix tissues were negative on staining for disease associated prion protein with identical methods as used in this study, though the spleen and lymph nodes gave positive results.[3] *PRNP* polymorphisms in sheep infected with scrapie also have a major influence on the incubation period and timing and distribution of disease associated prion protein in lymphoid tissues during the incubation period.[8]

A prolonged incubation period after infection with vCJD is likely to result in an asymptomatic carrier state (which cannot yet be identified), which represents a potential risk for horizontal transmission of vCJD infection by blood transfusion, blood products, or contaminated surgical instruments. These uncertainties further underline the need for continued surveillance of vCJD in the UK (including surveillance for subclinical or asymptomatic infection[9]), a requirement to continue to reduce the possibility of secondary iatrogenic transmission, and the inclusion of carrier states and susceptibility to vCJD infection in all *PRNP* codon 129 genotypes in future disease modelling.

Contributors: JWI (guarantor) and DAH were responsible for the prevalence study and the analysis of the results, including the selection of the cases for analysis, and drafted and modified the manuscript. MTB established the methods for DNA extraction and analysis, designed and executed the validation study, and drafted and modified the manuscript. KC and DH performed the DNA extraction on the test materials and in the validation study and modified the manuscript. MLeG, SL, DLR, and LMcC identified cases for the validation study and prepared the paraffin sections for DNA analysis and modified the manuscript.

Funding: The prevalence study was funded by the Department of Health (1216963 DAH; 1216982 JWI).

Competing interest: None declared.

Ethical approval: The prevalence study received approval from the South and West multi-centre research ethics committee (MREC reference 99/6/32) and for each of the centres included, appropriate local research ethics committee approval.

1 Hilton DA, Ghani AC, Conyers L, Edwards P, McCardle L, Penney M, et al. Accumulation of prion protein in tonsil and appendix: review of tissue samples. *BMJ* 2002;325:633-4.
2 Hilton DA, Ghani, A, Conyers L, Edwards P, McCardle L, Ritchie D, et al. Prevalence of lymphoreticular prion protein accumulation in UK tissue samples. *J Pathol* 2004;203:733-9.
3 Peden AH, Head MW, Ritchie DL, Bell JE, Ironside JW. Preclinical vCJD after blood transfusion in a PRNP codon 129 heterozygous patient. *Lancet* 2004;364:527-9.
4 Hainfellner JA, Liberski PP, Guiroy DC, Cervenakova L, Brown P, Gajdusek DC, et al. Pathology and immunohistochemistry of a kuru brain. *Brain Pathol* 1997;7:574-52.
5 McLean CA, Ironside JW, Alpers MP, Brown PW, Cervenakova L, Anderson RM, et al. Comparative neuropathology of Kuru with new variant Creutzfeldt-Jakob disease: evidence for strain of agent predominating over genotype of host. *Brain Pathol* 1998;8:429-37.
6 Hilton D, Sutak J, Smith MEF, Penney M, Conyers L, Edwards P, et al. Specificity of lymphoreticular accumulation of prion protein for variant Creutzfeldt-Jakob disease. *J Clin Pathol* 2004;57:500-2.
7 Goldfarb LG, Cervenakova L, Gajdusek DC. Genetic studies in relation to kuru: an overview. *Curr Mol Med* 2004;4:375-84.
8 Ersdal C, Ulvund MJ, Espenes A, Benestad SL, Sarradin P, Landsverk T. Mapping PrPSc propogation in experimental and natural scrapie with different PrP genotypes. *Vet Pathol* 2005;42:258-74.
9 Bird SM. Attributable testing for abnormal prion protein, database linkage and blood-borne vCJD risks. *Lancet* 2004;364:1362-4.

doi 10.1136/bmj.38804.511644.55

Prescribing for RITA

And so it ends—a decade in the training grade. The last rites performed with a final RITA (record of in training assessment): I'm finally grown up, the authorities deem. In fact, my consultant job starts tomorrow.

After 10 years with it, do I have positive suggestions for postgraduate training in the NHS? Of course, dozens, most involving workforce, reorganisation, and resources. But, as in life, the best tonics are free. I vote for a fresh culture that values and grows people. It is remarkable that NHS doctors deliver their high quality service for no immediate tangible gain. More extraordinary is that this work receives not a trace of the positive feedback and moral incentive that would be critical to the health of any comparable organisation.

I worked for some time in a prestigious institution of a more advanced healthcare system. What made their people tick? True, they had impressive buildings, state of the art technology, and good salary prospects; but, really, I think they were primarily driven by an ethos that valued excellence and individuality—initiated, fostered, and rewarded it. Right down to the artwork that lined the corridors—oversized portraits of the previous month's star employees, proud pictures of "graduating" trainees, plaques of senior faculty.

A bit over the top perhaps, but preferable to the anonymous passage of generations of juniors through Britain's many worthy hospitals. In addition, attitudes of derision towards the less skilled and suspicion of those who seem too good or creative are all too common. The end result? Blunted clones coming off an assembly line: competent, yes; extraordinary, no. Tragic for individuals and undesirable for a healthcare system that confronts extraordinary problems.

There, I've had my shout. Tomorrow I step into a new world, recognising that to change it is to change myself. I will not forget my morning dose of free tonic.

Giridhar P Kalamangalam *specialist registrar in neurology, Institute of Neurological Sciences, Southern General Hospital, Glasgow (g_kalamangalam@hotmail.com)*

別紙様式第 2-1
番号 13

医薬品
医薬部外品　　研究報告　調査報告書
化粧品

識別番号・報告回数			報告日	第一報入手日 2006 年 9 月 20 日	新医薬品等の区分 該当なし	厚生労働省処理欄
一般的名称	乾燥抗D（Rho）人免疫グロブリン		研究報告の公表状況	Future Virology 2006;1(5):659-674	公表国 日本	
販売名（企業名）	抗D人免疫グロブリン-Wf（ベネシス）					

研究報告の概要

1．血液製剤のプリオンに対するリスク（ｖCJDの発生状況）
・BSE の発生は少なくとも英国では減少しつつある。BSE に感染したウシからのvCJD伝播の危険性は低下傾向にある。他方、輸血によるヒトーヒト伝播の危険性は依然として続いている。

2．血漿分画製剤の安全対策
・血液中のプリオン検出のための高感度なスクリーニング方法は現在のところまだない。従って、今のところ、血漿分画製剤のプリオンに対する安全対策は、主として地理的条件によるドナーの排除と製造工程でのプリオンの除去である。

3．プリオン除去試験の留意点と試験結果
・プリオン除去のための個々の製造工程は、実際の製造条件を実験室での条件にスケールダウンさせ、確立されているスクレイピー株をモデル系として用いて通常は評価されている。血液中のプリオン蛋白の存在形態が不明なので、評価実験のためのスパイク材料としてのプリオンの調製方法は注意深く考慮しなければならない。
・プリオンアッセイする方法としては2 つの異なる方法がある。その2 つとはウエスタンブロッティングなどの方法でプリオンを検出するin vitro 試験と、サンプルを動物に接種することによって感染性プリオンを検出するin vivo 試験である。ごく少量のプリオン、例えばウエスタンブロッティング法の限界（例えば、抗体の不適切な使用など）によって陰性の結果を示すような量のプリオンであっても、サンプル中に感染性を検出することができることもある。従って、製造工程評価には、試験方法の間の相異を注意深く考慮しなければならない。
・製造工程条件の如何によって、除去パターンが類似したものであっても、その製造工程のプリオン除去能は変わりうる。従って、評価試験のデザインはきわめて重要である。各製造工程は個別に評価しなければならない。
・現在のところ、血漿分画製剤の製造工程のうち、エタノール分画、PEG 分画、カラムクロマトグラフィー、ウイルス除去膜およびデプスろ過膜でのろ過、その他がプリオン除去に有効であると考えられている。
・製造工程で安定してプリオン除去を行うためには、プリオン除去に寄与する複数の製造工程を組み合わせることが必要である。
・これらのことから、プリオンのスクリーニングと除去のための新しい技法の開発と製造工程評価方法の改良が切望される。

使用上の注意記載状況・その他参考事項等

2．重要な基本的注意
(1)略
1)略
2)現在までに本剤の投与により変異型クロイツフェルト・ヤコブ病（vCJD）等が伝播したとの報告はない。しかしながら、製造工程において異常プリオンを低減し得るとの報告があるものの、理論的な vCJD 等の伝播のリスクを完全には排除できないので、投与の際には患者への説明を十分行い、治療上の必要性を十分検討の上投与すること。

報告企業の意見	今後の対応
血漿分画製剤の製造工程におけるプリオン除去に関する総説論文であり、文献中の表に弊社が行なったプリオン除去試験結果を提示している。 これまで血漿分画製剤によってvCJDを含むプリオン病が伝播したとの報告はない。しかしながら、万一vCJD感染者の血漿が本剤の原料に混入した場合には、製造工程においてプリオンを低減し得るとの報告があるものの、製剤から伝播する可能性を完全には否定し得ない。そのため、弊社の血漿分画製剤の製造工程におけるTSE感染性低減に関する検証実験を加速し、自社データを早期に取得し、工程評価を行い、必要に応じて工程改善を実施する予定である。	vCJD に関連する情報については、今後も注視することとする。

REVIEW

Possible removal of prion agents from blood products during the manufacturing process

Mikihiro Yunoki†, Takeru Urayama & Kazuyoshi Ikuta

†Author for correspondence Infectious Pathogen Research Group, Hirakata Research Laboratory, Research & Development Division, Benesis Corporation, 2-25-1, Shodai-ohtani, Hirakata, Osaka 573-1153, Japan Tel.: +81 72 856 9260; Fax: +81 72 864 2341; yunoki.mikihiro@mk.m-pharma.co.jp

Blood products prepared from human blood theoretically risk contamination with infectious pathogens. Since recent reports now confirm the likely transmission of pathogenic prions through blood transfusion, effective measures to prevent transmission are required globally, although the prevalence of variant Creutzfeldt–Jakob disease outside of the UK is extremely low. Many studies evaluating the manufacturing process have been conducted for the potential removal of the prion protein from plasma derivatives. In this review, we discuss the possibility of removing prions via several processing steps, especially depth and virus-removal filtration. Through a discussion of the limitations and issues associated with such studies, we hope our review will be of help for better study design in the future.

Keywords: blood products, blood transfusion, bovine spongiform encephalopathy, clearance study, depth filter, prion, variant Creutzfeldt–Jakob disease, virus-removal filter

future medicine

The onset of illness in the first case of variant Creutzfeldt–Jakob disease (vCJD), which was published in 1996, occurred in early 1994. vCJD most probably results from the consumption of beef products contaminated by central nervous system tissue derived from bovines infected with bovine spongiform encephalopathy (BSE), which began in the UK sometime prior to 1986 [1]. The worldwide incidence of BSE and vCJD was approximately 190,000 animals and 185 patients (including 159 patients in the UK), respectively, in December 2005. In the UK, where the highest incidence of BSE and vCJD was reported, their peak incidences were observed in 1992 and 2000, respectively. Since these peaks, the incidence in the UK has decreased gradually [101]. There is also the possibility of continuing person-to-person transmission of vCJD through certain forms of healthcare (e.g., through surgery, blood transfusion or treatment with plasma products). Therefore, it is essential to maintain and promote active surveillance of vCJD and CJD (hereafter vCJD/CJD) to evaluate potential transmission by this route [2–4,102,103].

Since blood products are prepared from human blood, they may involve risks of contamination with infectious pathogens including pathogenic prions. Therefore, besides the measures for ordinary pathogens, effective measures implemented globally to prevent transmission of pathogenic prions (especially to prevent vCJD) are also required. Measures to prevent contamination by viruses/prions in plasma derivatives consist of donor plasma sourcing/screening and the elimination of viruses/prions during the manufacturing process. Currently, geographical deferral of blood donors before donation is the only method of identifying donors at higher risk for vCJD, since sensitive and rapid screening methods for prions in blood with the ability to handle many specimens have not currently been developed. The risks to a recipient from fractionated plasma products are probably less than from blood transfusion, not least owing to potential removal during the manufacturing process, as well as the volume of material to which an individual is exposed, which are likely to be important determinants of the level of risk [2]. However, the pooling of plasma donations and the large number of recipients from any given plasma pool complicates any calculations of residual risk.

Little is known regarding the native form of prion protein in blood, especially in plasma, although this information is essential for evaluating the safety of blood products. Under these circumstances, the regulatory agencies of several countries issued guidelines regarding measures to be taken to prevent or reduce the potential for prion transmission through pharmaceutical products. Manufacturers have implemented their measures according to these guidelines [5–7,104–107]. Recently, Brown reviewed prion infectivity in blood, prion removal by the manufacturing process and the current status of the development of prion-screening methods [8]. The removal of prions by partitioning during the manufacturing process is expected to be a practical and effective approach, particularly because effective methods for prion inactivation that are applicable to the manufacture of protein products, have not been developed to date. At present, the removal of prions by physical means is the main measure towards preventing prion contamination. There are numerous reports describing the partitioning and possible removal of prion

10.2217/17460794.1.5.659 ISSN 1746-0794

during the manufacturing process. Processes that possibly remove prions include fractionation, using ethanol and/or polyethylene glycol (PEG), and filtration through virus-removal and/or depth filters. Many studies have been performed on the efficacy of ethanol fractionation processes to remove prion and demonstrated similar removal ability, regardless of differences in study conditions and the research institutions. By contrast, studies on depth filtration revealed that the efficacy of depth filters to remove prions is highly dependent on the composition of the solution and/or characteristics of the filters. In this review, we discuss the possibilities and limitations of several manufacturing steps to remove prions using evaluation data from several manufacturers. We hope our discussion will be of help to determine better study design in the future. Cleaning (inactivation) of equipment should also be considered in parallel to the removal of prions during the manufacturing process. However, we will not discuss this area, and the reader is referred to Lee and colleagues who have already discussed this matter in details [9,10,108].

Study design for prion removal ability

Procedures for safety evaluation of plasma derivatives for prions are basically similar to those used for viruses. Regarding the virus clearance study (also termed virus validation study), the first regulatory guidance was issued by the European Community in 1989. Since then, manufacturers have performed virus clearance studies in accordance with this guideline as well as other related guidelines. On the other hand, the European Medical Agency issued guidelines regarding prion clearance in 2004 [104]. Their guideline was largely based on the concept of the virus validation guidelines [109], although care was taken to refer to such studies as investigational, as opposed to validation.

The following should be considered when performing prion-clearance studies.

Model agents

The main purpose of the clearance study is to assess and identify the manufacturing process(es) that can be considered to be effective in eliminating prions using various model agents, such as scrapie. Based on the results with model agents, the partitioning of specific human pathogenic agents, such as vCJD, can be speculated. In this sense, the purpose of the study is to evaluate the risk of the pathogen itself. However, if the pathogen in question is significantly different from the model agent, partitioning of the specific human pathogen may yield incorrect data. Therefore, for clearance studies and related studies, it is indispensable to carefully consider the possible differences between the pathogens in question and model agents.

Assay method

The detection of protease-resistant prion antigen (*in vitro* study) is performed as the first step (e.g., by western blotting [WB], and then, for certain process(es), an infectivity assay using animals (*in vivo* study) is also recommended. The *in vivo* assay remains the only possible option to confirm the quantitative infectivity titration of prions following inoculation of samples into animals. It should also be understood that, in some instances, there could be some discrepancy between *in vitro* and *in vivo* study results.

Simulation of manufacturing process

It may be impossible to exactly simulate all of the manufacturing process parameters on a laboratory scale. For experimental downscaling, it is impossible to use equipment and conditions that are identical to the actual manufacturing process. First, prion proteins are added intentionally, thereby changing the matrix. Second, the processes are downsized to laboratory scale. Therefore, in some instances, the best result that can be achieved is to approximate the behavior of the manufacturing process. Furthermore, it is important not to overestimate the prion removal ability of the manufacturing processes based on the data obtained under scaled-down conditions.

Native form of abnormal prion proteins

The native form of the abnormal prion proteins in blood is still unknown. Abnormal prion proteins in blood remain largely undetermined and may exist as various forms with different particle sizes or aggregation states. Therefore, the preparation method of the prion material used as a spiking agent for process evaluation studies would be an important factor.

Choice of spiking prion agent

Of the various prion diseases, vCJD is the primary concern for manufacturing plasma derivatives. It is difficult to use tissue samples taken from vCJD/CJD patients for the evaluation of manufacturing processes. Therefore, in general, laboratory strains of scrapie (e.g., 263K and ME7) and those of BSE (e.g., 301V) are used in place of vCJD materials [11–15].

Spiking materials are prepared from the brain of infected animals, and there are several methods of preparation. Brain homogenate (BH) has been widely used for a long time, because BH is easy to prepare and contains a high titer of infectivity. However, the uniformity of particle size is not ideal for evaluation purposes. The microsomal fraction (MF) is partially purified from BH, and the titer of MF may be slightly lower than BH. In addition, caveolae-like domains and semipurified scrapie prion protein (purified fibrils) may also be used in spiking studies. The partitioning of abnormal prion protein prepared by different preparations behaves in a similar manner, with the exception of purified fibrils [16–18]. Although the appropriateness of the materials used for spiking experiments was described in reported studies, there has been no discussion of their particle size.

In 2003, Yunoki reported that the particle size of MF used as spiking material was 800 nm on average, and that the particle size of MF fell to less than 220 nm through high-power sonication or detergent treatment [19]. At present, researchers tend to add steps such as sonication, detergent treatment and prefiltration in order to prepare MF or BH for spiking studies. However, discussion regarding the appropriateness of spiking materials is limited because the status of the abnormal prion protein in blood is not currently clear. Various preparation methods have been used in the above reports. Therefore, it is necessary to carefully consider such preparation methods used in individual reports in order to evaluate the removability of prion agents during the process.

As our knowledge regarding the form and characteristics of abnormal prion protein in blood accumulates, these problems are expected to be resolved. A new preparation method utilizing exosomes might be proposed because a recent report described that prions also exist in association with exosomes [20]. In addition, spiking materials derived from cultured cells producing abnormal prion protein may become one possible source for spiking prion material [21]. If strains of vCJD/CJD with a high titer are prepared in cultured cells, it would become possible to use such materials as spiking agents.

Evaluation methods

To estimate the prion levels in samples, two different methods are used: one to detect abnormal prion protein in samples by WB, conformation-dependent immunoassay or enzyme-linked immunosorbent assay (*in vitro* study) [16,22–24]; and the other to detect pathogenic prion protein by inoculating animals with the samples (*in vivo* study). Although WB is widely used, assay conditions are different in every laboratory and there is no standard protocol for the assay. In general, samples taken from manufacturing processes contain plasma proteins at a high level, which sometimes disturb the specific detection of a small amount of prion by WB. To avoid these problems, optimization of assay conditions and/or adjustment of pretreatment conditions for each sample are necessary. Due to these assay variables, the sensitivity of the assay not only differs in every laboratory, but even from sample to sample. When an identical sample is used for comparison, WB generally gives a lower sensitivity than the *in vivo* method. To improve the sensitivity and specificity of WB, several methodologies have been performed; for example, the elimination of plasma proteins that disturb the assay, by heating at 80°C before proteinase K treatment, followed by ultracentrifugation to concentrate prion [25].

For viruses, it is required that clearance studies are performed following the detection of infectivity of process samples as an indicator. However, for prions, according to the guidelines [104,105,109], infectivity experiments *in vivo* are not always required for processes where the relationship between *in vivo* and *in vitro* results has been established. For processes where the relationship is unknown, such as new processes, it may be necessary to check the infectivity of samples *in vivo* following initial testing of samples *in vitro*.

In general, experimental conditions for the detection of infectivity using animals differ at every institution. Even if animals are the same species and age, the amount of inoculums given to the animals and/or incubation period of the animals after inoculation may be different. Symptoms of prion diseases are monitored by clinical signs during the incubation period in animals, although the monitoring procedure may also differ at each institution. Some institutions monitor abnormal behavior only, whereas other institutions use a scoring system for monitoring. However, such observation of clinical signs may not be regarded as a definitive indicator of disease. Classically, histopathology has been used to confirm disease lesions in brain samples taken from infected animals [26]. Similarly, different criteria in pathological examinations are used by institutions to determine prion lesions. Some institutions judge prion diseases by

the existence of vacuoles only. Some other institutions use their own scoring system using several factors, such as spongiform vacuolization, gliosis or amyloid plaques in the lesion sites. The above judgments are sometimes problematic because these pathological observations must be performed by experienced investigators. To obtain more knowledge regarding the diagnosis of prion diseases, see the general review by Kretzschmar [27]. To avoid the above problems, rather than pathological evaluation, a recent trend is the *in vitro* detection of abnormal prion protein using BHs from inoculated animals by immunological procedures, such as WB.

Lee and colleagues published a report in 2004 regarding the relationship between *in vitro* and *in vivo* results [28]. Their data demonstrate that the partition of prion antigen in individual process samples detected by WB was consistent with that of infectious prions observed *in vivo*. These results suggest that it may be possible to evaluate prion partitioning during the manufacturing process by *in vitro* study using only WB. However, they also demonstrated that, in some cases, infectivity remains in a sample where the amount of abnormal prion protein is less than the limit of WB. One possibility for the phenomenon is the inappropriate use of antiprion antibodies for WB. Based on their results, the data obtained by *in vitro* study should be evaluated with the possibility that such study may have limitations for the detection of prion agents.

Cell culture to persistently maintain the infectious prion protein has been widely reported, and the development of a cell culture system for the quantitative detection of prion infectivity is now underway [21,29,30]. In the future, if a new assay system using a cell culture system demonstrates the same sensitivity as animal studies and good correlation, experiments to detect infectivity may be switched from animal systems to cell culture studies, as has occurred for some viruses used for virus validation studies.

Evaluation of the major manufacturing processes for prion removal

Concept for evaluation of manufacturing processes

Over the last 10 years, many reports have been published on prion removal during the manufacturing processes of plasma derivatives. To reassess these reports today, we must consider the technical background of the studies (discussed earlier). Before the European Medicines Agency published a statement in 2004, the strategy for establishing the study design was not as clear [105].

The log reduction factor of prion by a certain manufacturing process is often misunderstood as representing an unconditional absolute value; however, this factor is merely one of the indices for process evaluation. Therefore, based on the comprehensive grasp of all information, judgment should be made whether the process in question is effective, partially effective or ineffective for prion removal by individual manufacturing steps.

Several procedures are expected to remove abnormal prion protein. Fractionation with ethanol, PEG and glycine, and filtration with virus-removal and depth filter have been widely investigated, and many reports have been published on these steps (described later).

Fractionations during plasma protein purification steps

Many studies have already been performed on ethanol fractionation. Details of ethanol fractionation and prion partitioning during the manufacturing process have been described in several articles [31,32,104,105]. Among the ethanol fractionation processes, Fraction II + III, Fraction III and Fraction IV processes exhibited significant partitioning (Table 1). These are considered to be effective prion removal processes. For PEG and glycine fractionations, several studies have also been reported, as summarized in Table 2. PEG fractionation processes, including 8 and 11.5%, demonstrated good partition and are regarded as effective prion removal processes, such as ethanol fractionation processes, whereas glycine fractionation demonstrated less effective removal.

For column chromatography, various kinds of columns demonstrated a different tendency to partition prion protein (Table 3). All of the column chromatography processes reviewed here are not implemented specifically for the removal of prions, but for purification of the plasma protein of interest. In this sense, the removal of prion with these column chromatography processes is, if anything, a secondary effect. Therefore, the factors and/or parameters that are necessary to purify plasma proteins will differ from those for prion removal.

Virus-removal filters

Virus-removal filters were developed exclusively for effective removal of viruses during manufacturing processes, and had pore sizes of approximately

Table 1. Removal of prion by ethanol fractionation*.

Process (condition)	Spiking agent	Spike source	Before	Filtered (supernatant)	Paste (precipitate)	Clearance/reduction		Method	Ref.
						Filtered	*Paste*		
Cryoseparation	Human PrP^{vCJD}	BH	3.9	3.0	3.6	0.9	0.3	WB	[37]
	Human PrP^{sCJD}	BH	3.7	2.8	2.7	0.9	1.0	WB	
	Human PrP^{GSS}	BH	4.5	3.5	3.7	1.0	0.8	WB	
	Sheep PrP^{Sc}	BH	3.0	2.0	2.5	1.0	0.5	WB	
	Hamster PrP^{Sc} 263K	BH	5.9	4.7	5.3	1.2	0.6	WB	
	Hamster PrP^{Sc} Sc237	BH	2.3	2.0	2.1	0.3	0.2	CDI	[16]
		MF	3.4	3.2	2.9	0.2	0.5	CDI	
		CLDs	2.8	2.4	2.6	0.4	0.2	CDI	
		Purified	3.8	1.4	3.4	2.4	0.4	CDI	
	Hamster PrP^{Sc} 263K	TR	8.1	NA	6.0	NA	2.1	BA	[17]
	Mouse PrP^{GSS}	Blood	+ve	NA	+ve	NA	NA	BA	
	Hamster PrP^{Sc} 263K	BH	7.8	6.8	7.2	1.0	0.6	BA	[28]
			2.9	1.9	2.6	1.0	0.3	WB	
	Hamster PrP^{Sc} 263K	MF	ND	ND	ND	<1.0	1.0	WB	[18]
Fraction I (8% ethanol)	Hamster PrP^{Sc} Sc237	BH	4.4	3.5	4.3	0.9	0.1	CDI	[16]
		MF	4.4	3.5	4.4	0.9	0.0	CDI	
		CLDs	3.7	3.0	3.7	0.7	0.0	CDI	
		Purified	4.1	1.0	3.9	3.1	0.2	CDI	
Fraction II + III (20% ethanol)	Hamster PrP^{Sc} 263K	BH	8.5	2.5	8.5	6.0	0.0	BA	[28]
			4.9	≤0.2	5.3	≥4.7	0.0	WB	
Fraction II + III (25% ethanol)	Hamster PrP^{Sc} Sc237	BH	4.1	0.5	4.1	3.6	0.0	CDI	[16]
		MF	4.9	1.8	4.9	3.1	0.0	CDI	
		CLDs	3.9	0.8	3.8	3.1	0.1	CDI	
		Purified	4.6	0.6	4.3	4.0	0.3	CDI	
Fraction I + II + III (19% ethanol), including filter aid	Hamster PrP^{Sc} 263K	BH	7.0	4.8	ND	2.2	NA	BA	[38]
			ND	ND	ND	3.8	NA	WB	

*Values given are expressed in log_{10} form. ‡Clearance was calculated by subtracting the effluent titers from the precipitate titers. §Including depth filtration. ¶Yunoki et al. Unpublished Data.
BA: Bioassay (in vivo study); BH: Brain homogenate; BSE: Bovine spongiform encephalopathy; CDI: Conformation-dependent immunoassay; CLD: Caveolae-like domain; GSS: Gerstmann–Sträussler–Scheinker syndrome; MF: Microsomal fraction; NA: Not applicable; ND: Not determined; PrP: Prion protein; Sc: Scrapie; sCJD: Sporadic Creutzfeldt–Jakob disease; sMF: Sonicated MF; TR: Trypsin-treated minced brain; vCJD: Variant Creutzfeldt–Jakob disease; WB: Western blotting (in vitro study).

Table 1. Removal of prion by ethanol fractionation* (cont.).

Process (condition)	Spiking agent	Spike source	Before	Filtered (supernatant)	Paste (precipitate)	Clearance/reduction		Method	Ref.
						Filtered	*Paste*		
Fraction I + II + III (20% ethanol), evaluate from plasma	Hamster PrP^{Sc} 263K	TR	8.1	NA	6.1	NA	2.0	BA	[17]
	Mouse PrP^{GSS}	Blood	+ve	NA	+ve	NA	NA	BA	
Fraction I + II + III (21% ethanol)	Hamster PrP^{Sc} 263K	MF	ND	ND	ND	1.3	<1.0	WB	[18]
Fraction III (17% ethanol)	Hamster PrP^{Sc} 263K	BH	ND	2.0	7.3	5.3[†]	0.0[†]	BA	[28]
			4.3	0.0	4.3	≥4.3	0.0	WB	
Fraction I + III (12% ethanol)	Hamster PrP^{Sc} 263K	BH	6.8	3.3	ND	3.5	NA	BA	[38]
			ND	ND	ND	4.5	NA	WB	
Fraction I + III (12% ethanol)	Mouse PrP^{BSE} 301V	MF	6.1	4.0	6.0	2.1	0.1	BA	[18,39]
	Hamster PrP^{Sc} 263K	MF	ND	ND	ND	≥3.7	NA	WB	
Fraction IV (38% ethanol), high prion spiked	Hamster PrP^{Sc} Sc237	BH	4.1	0.9	3.4	3.2 (≥4.1)[§]	0.7	CDI	[16]
		MF	4.5	1.1	4.5	3.4 (≥4.5)[§]	0.0	CDI	
		CLDs	4.1	0.9	3.8	3.2 (≥4.1)[§]	0.3	CDI	
		Purified	4.6	2.4	4.4	2.2 (≥4.6)[§]	0.2	CDI	
Fraction IV (38% ethanol), low prion spiked	Hamster PrP^{Sc} Sc237	MF	3.7	0.8	3.5	2.9 (≥3.7)[§]	0.2	CDI	[16]
		CLDs	3.0	0.0	3.0	≥3.0 (≥3.0)[§]	0.0	CDI	
		Purified	3.2	0.0	2.8	≥3.2 (≥3.2)[§]	0.4	CDI	
Fraction IV (35% ethanol)	Hamster PrP^{Sc} 263K	MF	ND	ND	ND	≥3.0	NA	WB	[18]
Fraction IV (40% ethanol)	Hamster PrP^{Sc} 263K	BH	7.0	4.0	ND	3.0	NA	BA	[38]
			ND	ND	ND	5.0	NA	WB	
Fraction IV_1	Hamster PrP^{Sc} 263K	BH	8.9	5.2	7.5	3.7	1.4	BA	[28]
			4.2	0.0	4.2	≥4.2	0.0	WB	
Fraction IV_4	Hamster PrP^{Sc} 263K	BH	7.6	3.0	7.2	4.6	0.4	BA	[¶]
			4.2	≤0.1	4.0	≥4.1	0.2	WB	
Fraction IV	Hamster PrP^{Sc} 263K	sMF	3.6	<0.6	3.8	≥3.0	0.0	WB	[¶]
Fraction IV_1 + IV_4 (40% ethanol) evaluate from plasma	Hamster PrP^{Sc} 263K	TR	8.1	NA	3.9	NA	4.2	BA	[17]
	Mouse PrP^{GSS}	Blood	+ve	NA	-ve	NA	NA	BA	

*Values given are expressed in log_{10} form. [†]Clearance was calculated by subtracting the effluent titers from the precipitate titers. [§]Including depth filtration. [¶]Yunoki et al. Unpublished Data.
BA: Bioassay (in vivo study); BH: Brain homogenate; BSE: Bovine spongiform encephalopathy; CDI: Conformation-dependent immunoassay; CLD: Caveolae-like domain; GSS: Gerstmann–Sträussler–Scheinker syndrome; MF: Microsomal fraction; NA: Not applicable; ND: Not determined; PrP: Prion protein; Sc: Scrapie; sCJD: Sporadic Creutzfeldt–Jakob disease; sMF: Sonicated MF; TR: Trypsin-treated minced brain; vCJD: Variant Creutzfeldt–Jakob disease; WB: Western blotting (in vitro study).

Table 2. Removal of prion by polyethylene glycol, glycine and caprylate precipitation*.

Process (sample)	Spiking agent	Method	Before	Filtered (supernatant)	Paste (precipitate)	Clearance/reduction		Method	Ref.
						Filtered	*Paste*		
3% PEG (cryoprecipitate)	PrP^{vCJD}	BH	4.0	2.1	4.0	1.9	0.0	WB	[37]
	PrP^{sCJD}	BH	3.7	1.5	3.7	2.2	0.0	WB	
	PrP^{GSS}	BH	5.0	3.0	5.0	2.0	0.0	WB	
	Sheep PrP^{Sc}	BH	4.0	2.3	4.0	1.8	0.0	WB	
	Hamster PrP^{Sc} 263K	BH	6.3	4.1	6.1	2.2	0.2	WB	
3% PEG (cryoprecipitate)	Hamster PrP^{Sc} 263K	BH	7.2	5.0	7.2	2.2	0.0	BA	[28]
			5.2	2.2	4.9	3.0	0.3	WB	
8% PEG (IVIG)	Hamster PrP^{Sc} 263K	sMF	2.5	<0.1	3.2	≥2.4	0.0	WB	¶
		MF	2.5	<0.1	2.5	≥2.4	0.0	WB	
		sMF	Prob. +ve	Prob. +ve	ND	NA	NA	BA	
11.5% PEG (Fraction IV_1 precipitate)	PrP^{vCJD}	BH	4.0	0	4.2	≥4.0	0.0	WB	[37]
	PrP^{sCJD}	BH	3.0	0	2.9	≥3.0	0.1	WB	
	PrP^{GSS}	BH	4.0	0	4.0	≥4.0	0.0	WB	
	Sheep PrP^{Sc}	BH	3.5	0	3.5	≥3.5	0.0	WB	
	Hamster PrP^{Sc} 263K	BH	5.8	0	5.7	≥5.8	0.1	WB	
11.5 % PEG (Fraction IV_1 precipitate)	Hamster PrP^{Sc} 263K	BH	ND	≤1.1	6.5	≥5.4‡	0.0‡	BA	[28]
			4.9	0	4.6	≥4.9	0.3	WB	
Glycine (cryoprecipitate)§	Hamster PrP^{Sc} Sc237	MF	3.1	1.4	2.3	1.7	0.8	CDI	[16]
		Purified	3.8	0.5	3.1	3.3	0.7	CDI	
SD+8%Glycine (fibrinogen)	Hamster PrP^{Sc} 263K	sMF	3.0	2.7	3.5	0.3	0.0	WB	¶
SD+15%Glycine (Factor VIII)	Hamster PrP^{Sc} 263K	MF	2.5	2,2	1.5	0.3	1.0	WB	¶
		sMF	2.5	2.9	1.5	0.0	1.0	WB	
Caprylate precipitation/cloth filtration (Fraction II + III suspension/IVIG)	Hamster PrP^{Sc}	ND	ND	ND	ND	ND	2.9	WB	[40]
		ND	ND	ND	ND	ND	3.3	BA	

Values given are expressed in log_{10} form. ‡Clearance was calculated by subtracting the effluent titers from the precipitate titers. §Cryoprecipitate after $Al(OH)_3$ adsorption. ¶Yunoki et al. Unpublished Data. BA: Bioassay (in vivo study); BH: Brain homogenate; CDI: Conformation-dependent immunoassay; GSS: Gerstmann-Sträussler–Scheinker syndrome; IVIG: Intravenous immunoglobulin; MF: Microsomal fraction; Prob. +ve: Probable positive; PrP: Prion protein; NA: Not applicable; ND: Not determined; Sc: Scrapie; sCJD: Sporadic Creutzfeldt–Jakob disease; sMF: Sonicated MF; vCJD: Variant Creutzfeldt-Jakob disease; WB: Western blotting (in vitro study).

Table 3. Removal of prion by column steps*.

Process	Sample	Spiking agent	Method	Before	Pass	Eluate	Retained	Reduction (clearance) for product fraction	Method	Ref.
DEAE Tyoperl 650M	SD contained Factor VIII	Hamster PrP^{Sc} 263K	MF	ND	ND	ND	ND	≥3.5‡ 3.1§	WB	[18]
		Mouse PrP^{BSE} 301V	MF	8.7	ND	<5.9‡ 6.1§	7.6	≥2.9‡2.7§	BA	[41]
DEAE-sepharose	Factor IX	Hamster PrP^{Sc} 263K	MF	ND	ND	ND	ND	3.0	WB	[18]
Heparin-sepharose	SD contained Factor IX	Hamster PrP^{Sc} 263K	MF	ND	ND	ND	ND	1.4	WB	[18]
S-sepharose	SD contained thrombin	Hamster PrP^{Sc} 263K	MF	ND	ND	ND	ND	2.9	WB	[18]
MoAb	Factor IX	Hamster PrP^{Sc} 263K	dMF¶	3.7	3.7	1.3	NA	2.4	WB	#

Values are expressed in log_{10} form. ‡Fibrinogen fraction. §Factor VIII fraction. ¶SD (0.3% TNBP and 1%Tween 80) treated. #Yunoki et al. Unpublished Data.

BA: Bioassay (in vivo study); BSE: Bovine spongiform encephalopathy; DEAE: Diethylaminoethyl; dMF: Detergent-treated MF; MF: Microsomal fraction; MoAb: Monoclonal antibody; NA: Not applicable; ND: Not determined; PrP: Prion protein; Sc: Scrapie; SD: Solvent and detergent; WB: Western blotting (in vitro study)

15–35 nm depending on the filter type. For example, 15N (15±2 nm), 20N (19±2 nm) and 35N (35±2 nm) of Planova filters (Asahi Kasei Medical Co., Ltd., Tokyo, Japan); DV20 (>3-logs reduction of virus particles >20 nm in diameter, and >6-logs reduction of virus particles >50 nm in diameter); and DV50 (>6-logs reduction of virus particles >50 nm in diameter) of DV filters (Pall Co., NY, USA); and Viresolve70 (filtration of molecules with <70 kDa) (Millipore Co., Billerica, MA, USA). Although the filters were originally used for the removal of viruses, it is expected that they may also be applicable for the removal of prions. To date, only a few reports have been published on the prion removal capacity of virus-removal filters. In this review, we refer only to reports in which the name of the filter is specified (Table 4).

In 2005, Silveira and colleagues reported that prion protein particles with a particle size of 17–27 nm retain prion infectivity in an *in vivo* study [33]. However, prion particles can be much larger and 17–27 nm particles appear to be at the low end of size distribution [34]. Using this estimated size of the minimum infectious particle, we can infer useful information from the study results on parvovirus partitioning, because the particle size (20–26 nm) of the virus is similar to that of prions. Virus-removal filters with a nominal pore size of 15 nm can remove canine parvovirus and parvovirus B19 (B19) effectively [19,35,36]. Therefore, virus removal filters with 15-nm pore size should be useful for prion removal. We obtained evidence that scrapie prion could also be removed effectively by a 15-nm filter, at least when assayed using WB, although infectivity in the filtrate remained when we inoculated hamsters [Yunoki and colleagues, Unpublished Data].

However, many plasma products cannot be filtered with this filter. In fact, even for the evaluation of virus-removal filters for prion removal, there are several technical issues to be noted. Most of the problems associated with the 15-nm filter occur due to clogging of the filter by spiking materials. Clogging often prolongs the filtration time and renders the filter unable to process the required loaded amount per unit surface area of the filter (termed deviation).

Table 4. Removal of prion by virus filters*.

Process	Sample	Spiking agent	Method	Before	Filtered	Retained	Clearance/reduction		Method	Ref.
							Filtered	*Retained*		
VireSolve180 (Millipore)	0.5% immunoglobulin	Hamster PrP^{Sc} 263K	dsBH**	6.4/6.9/6.9	<3.9/<3.9/<3.9	5.9/6.4/5.9	≥2.5/≥3.0/ ≥3.0	0.5/0.5/ 1.0	WB	[42]
Planova 75N (Asahi)	PBS	Hamster PrP^{Sc} 263K	MF	3.5/4.2	<1.0/<1.0	ND/ND	≥2.5/≥3.2	NA/NA	WB	[19]
			sMF	4.2/4.2	2.4/2.4	ND/ND	1.8/1.8	NA/NA	WB	
Planova 35N (Asahi)	2% albumin	Mouse PrP^{Sc} ME7	BH	8.13	3.20	ND	4.93	NA	BA	[43]
			dBH‡	7.32	5.71	ND	1.61	NA	BA	
	PBS	Hamster PrP^{Sc} 263K	MF	3.5/4.2	<1.0/<1.0	ND/ND	≥2.5/≥3.2	NA/NA	WB	[19]
			sMF	4.2/4.2	<1.0/<1.0	ND/ND	≥3.2/≥3.2	NA/NA	WB	
	IVIG	Hamster PrP^{Sc} 263K	sMF	3.2/2.5	0.8/0.8	ND/ND	2.4/1.7	NA/NA	WB	
	Haptoglobin	Hamster PrP^{Sc} 263K	sMF§§	2.4	<1.0	ND	≥1.4	NA	WB	##
Planova 20N (Asahi)	IVIG	Hamster PrP^{Sc} 263K	sMF	6.8/6.8	4.8/4.3	ND/ND	2.0/2.5	NA/NA	WB	##
	Haptoglobin		dsMF‡‡	6.7/6.1	4.8/4.7	ND/ND	1.9/1.4	NA/NA	WB	
Planova 15N (Asahi)	2% albumin	Mouse PrP^{Sc} ME7	BH	8.13	<2.26	ND	>5.87	NA	BA	[43]
			dBH‡	7.32	<3.11	ND	>4.21	NA	BA	
	PBS	Hamster PrP^{Sc} 263K	MF	3.5/4.2	<1.0/<1.0	ND/ND	≥2.5/≥3.2	NA/NA	WB	[19]
			sMF	4.2/4.2	<1.0/<1.0	ND/ND	≥3.2/≥3.2	NA/NA	WB	
	Antithrombin III	Hamster PrP^{Sc} 263K	dMF§	3.1/3.1	0.0/0.0	ND/ND	≥3.1/≥3.1	NA/NA	WB	
			sMF§§	3.6	<0.8	NA	≥2.8	NA	WB	##
				Prob. +ve	Prob. +ve	NA	NA	NA	BA	
	Thrombin	Hamster PrP^{Sc} 263K	dsMF†‡	3.7/3.7	<0.2/<0.2	ND/ND	≥3.5/≥3.5	NA	WB	##
Planova 10N (Asahi)	2% albumin	Mouse PrP^{Sc} ME7	dBH‡	7.32	<3.52	ND	>3.80	NA	BA	[43]
DVD + DV50 + DV20 (Pall)	Globulin	Human CJD^{Res}	BH	ND	ND	ND	3.0–3.3¶ >2.3¶¶ >1.6#	NA	WB	[44]

**Values given are expressed in log_{10} form. **Sonicated BH including 0.1% lysolecitin and followed by 0.45-0.22-0.1 µm serially filtered. ‡Including 0.5% sarcosyl. ‡‡SD (0.3% TNBP and 1% Tween 80) treated and followed by sonication. §Including 0.1% sarcosyl. §§0.22 µm filtered. ¶1:10 BH spiked. ¶¶1:100 BH spiked. #1:500 BH spiked. ##Yunoki et al. Unpublished Data.*

BA: Bioassay (in vivo study); BH: Brain homogenate; CJD: Creutzfeldt–Jakob disease; dBH: Detergent-treated BH; dMF: Detergent-treated MF; dsBH: Detergent treated and sonicated BH; dsMF: Detergent-treated and sonicated MF; IVIG: Intravenous immunoglobulin; MF: Microsomal fraction; NA: Not applicable; ND: Not determined; PBS: Phosphate buffered saline; Prob. +ve: Probable positive; PrP: Prion protein; Res: Protease resistant; Sc: Scrapie; sMF: Sonicated MF: WB: Western blotting (in vitro study).

Such deviations from standard manufacturing conditions should be carefully considered, without overestimation, for the acceptability of such studies. In such instances, the smaller pores tend to clog first, which diverts more of the flow through the larger pore sizes, thereby changing the effective pore size of the filter. For the purposes of risk assessment, it appear to be appropriate to assume that, even with a 15-nm filter, leakage of only a small amount of prion (less than the limit of *in vitro* detection methods) may occur, as often found in parvovirus studies.

Process evaluation of virus-removal filters must be performed considering the above points. Since the published study design for process evaluation of prion removal is often unclear, reports must be reassessed carefully to exclude the possibility that prion clearance has been adversely affected. It is generally accepted that the basic principle of virus-removal filtration is size exclusion. Depending on the filtration conditions, the performance of the filters may vary. At present, the 15-nm filter is the most effective for prion removal, although filters with a pore size of 20 nm or more can also remove prions to some extent. However, it should be understood that, in theory, all filters may leak infectious prions into the filtrate. Owing to clogging and other problems, the percentage spiking may need to be reduced in many cases. Consequently, the removal factor tends to be lower, which should also be considered carefully.

In the future, it may be necessary to develop virus-removal filters with smaller pore sizes. However, smaller pore size may also be more problematic because not only contaminants but also the desired plasma protein may be captured by the filter. Therefore, some other measures, such as improvement of filtration efficiency with 15-nm pore size filters, or identifying suitable filtration conditions for larger pore filters (e.g., by inducing prion aggregates at low pH prior to filtration) may become important.

Depth filters

The basic principle of depth filtration is to remove and/or capture impurities by filtration through a multilayered matrix structure. Pore sizes of depth filters usually range from 0.1 to 5.0 µm. Some improved filters are electrically charged to capture impurities more efficiently. Depth filtration was originally introduced for the clarification of protein solutions and, thus, was not intended specifically for prion removal. Therefore, contaminating prion agents are removed as a secondary effect during purification of the desired protein. Considering the pore size of depth filters, the filtration mechanism for prion removal cannot be simply explained only by size exclusion, because the charge of the depth filter could also be involved in prion removal. However, certain conditions may result in significant prion aggregation (e.g., low pH), and under such conditions, removal by size exclusion may be the primary mechanism of removal. To date, only a small number of reports have been published on prion removal by depth filtration. The results of the studies are summarized in Table 5 (reports where the name of the filter was not specified are not included).

According to a report that even prion particles of 17–27 nm in diameter still remain infective [33], such infectious prions should theoretically pass through depth filters. However, a number of reports highlight that, in some cases, abnormal prion was actually removed by depth filtration. Even with an identical filter, filtration efficiency varies significantly depending on filtrating conditions. Therefore, depth filtration cannot guarantee consistent prion removal in each instance, but rather conditions may need to be optimized for each product. Thus, in actual manufacturing, conditions for depth filtration must be defined with strict process controls in order to ensure effective prion removal. Furthermore, any evaluation study of the process should be designed very carefully while considering the processing conditions. The correlation of prion partitioning via depth filtration in model systems and vCJD/CJD systems remains to be confirmed.

There are several technical problems to be noted when we evaluate depth filtration. The biggest problem is that the mechanism of prion removal has not currently been clarified. Size exclusion alone cannot explain the mechanism to remove prions by depth filters. Electrically charged matrices may adsorb prions, but this has not been investigated in detail. Therefore, process evaluation for depth filtration may require very careful design, since there is a possibility of behavioral differences between model and vCJD/CJD systems during filtration. In addition, it is difficult to obtain depth-filter materials of uniform quality. This particular problem must be improved for the usefulness of the depth filtration process to remove prions at the manufacturing level.

In general, from the results of previous studies, depth filtration may be effective, to some extent, for prion removal, as Foster and coworkers

Table 5. Removal of prion by depth filters*.

Step	Sample	Spiking agent	Method	Before	Filtered	Retained	Clearance/reduction		Method	Ref.
							Filtered	*Retained*		
Seitz Supra P80‡ (Pall)	Supernatant of 8% ethanol precipitation	Hamster PrP^{Sc} Sc237	BH	3.5	3.4	ND	0.1	NA	CDI	[16]
			MF	3.5	3.6	ND	0.0	NA	CDI	
			CLDs	3.0	3.0	ND	0.0	NA	CDI	
			Purified	1.0	1.0	ND	0.0	NA	CDI	
	Supernatant of 38% ethanol precipitation		BH	0.9	0.0	ND	≥0.9	NA	CDI	
			MF	1.1(H) 0.8(L)	0.0(H) 0.0(L)	ND	≥1.1(H) ≥0.8(L)	NA	CDI	
			CLDs	0.9	0.0	ND	≥0.9	NA	CDI	
			Purified	2.4	0.0	ND	≥2.4	NA	CDI	
AP 20 (Millipore)	Supernatant I + III	Mouse PrP^{BSE} 301V	MF	7.0(H) 4.0(L)	4.6(H) 3.4(L)	ND	2.4(H) 0.6(L)	NA	BA	[39]
		Hamster PrP^{Sc} 263K	MF	ND	ND	ND	<1.0(L)	NA	WB	
	Supernatant I + III	Hamster PrP^{Sc} 263K	MF	ND	ND	ND	<1.0	NA	WB	[18]
Seitz KS 80 (Pall)	Supernatant I + III (AP20 filtered described as above)	Mouse PrP^{BSE} 301V	MF	6.3(H) 4.6(L)	≤3.2(H) ≤3.2(L)	ND	≥3.1(H) ≥1.4(L)	NA	BA	[39]
	Resuspended fraction V	Hamster PrP^{Sc} 263K	MF	ND	ND	ND	≥4.9	NA	WB	[18]

Note: data are referred from indicated reports and partially altered.

**Values given are expressed in log_{10} form. ‡Sonicated BH including 0.1% lysolecitin and followed by 0.45-0.22-0.1 µm serially filtered. The spiking agent was added before precipitation and CDI was performed after precipitation and after depth filtration. §Salt strip 1 M NaCl followed by 2 M. ¶0.22 µm-filtered prior depth filter. ‖0.22 µm filtered. ‖‖Yunoki* et al. *Unpublished Data.*

*BA: Bioassay (*in vivo *study); BH: Brain homogenate; BSE: Bovine spongiform encephalopathy; CDI: Conformation-dependent immunoassay; CDLs: Caveolae-like domains; dsBH: Detergent treated and sonicated BH; E: Early filtrate; H: High titer of prion on assay of spiked feed stock; IVIG: Intravenous immunoglobulin; L: Low titer of prion on assay of spiked feed stock; La: Late filtrate; M: Middle filtrate; MF: Microsomal fraction; NA: Not applicable; ND: Not determined; PrP: Prion protein; Sc: Scrapie; sMF: Sonicated MF; WB: Western blotting (*in vitro *study).*

Table 5. Removal of prion by depth filters* (cont.).

Step	Sample	Spiking agent	Method	Before	Filtered	Retained	Clearance/reduction		Method	Ref.
							Filtered	*Retained*		
Seitz K200P (Pall)	Resuspended fraction II	Hamster PrP^{Sc} 263K	MF	ND	ND	ND	≥2.8	NA	WB	[18]
Delipid 1 (Cuno)	Clarified fraction V suspension	Hamster PrP^{Sc} 263K	MF	ND	ND	ND	2.3	NA	WB	[18]
Zetaplus Delipid Plus (Cuno)	IVIG	Hamster PrP^{Sc} 263K	sMF	2.8	2.5	ND	0.3	NA	WB	##
			sMF#	4.1	2.7	ND	1.4	NA	WB	
			MF	2.8	1.1	ND	1.7	NA	WB	
Zetaplus 30LA (Cuno)	IVIG	Hamster PrP^{Sc} 263K	sMF	2.8	<0.4	ND	≥2.4	NA	WB	##
			MF	3.5	<0.4	ND	≥3.1	NA	WB	
Zetaplus 90SP (Cuno)	Supernatant III	Hamster PrP^{Sc} 263K	dsBH‡	7.9	<2.7(E) 4.2(M) 4.3(La)	7.4§	>3.3	0.5	WB	[45]
				7.1 <4.1¶	<2.7(E) <2.1(M) <2.0(La)	<3.6§	NA	NA	WB	
	Tris-buffered saline			7.0	6.2(E) 6.7(M) 6.0(La)	4.8§	0.1	2.2	WB	
Zetaplus 90LA (Cuno)	Clarified fraction V suspension	Hamster PrP^{Sc} 263K	sMF	3.5	<0.4	ND	≥3.1	NA	WB	##
			sMF#	4.5	<0.9	ND	≥3.6	NA	WB	
			MF	3.5	<0.4	ND	≥3.1	NA	WB	

Note: data are referred from indicated reports and partially altered.

**Values given are expressed in log_{10} form. ‡Sonicated BH including 0.1% lysolecitin and followed by 0.45-0.22-0.1 µm serially filtered. The spiking agent was added before precipitation and CDI was performed after precipitation and after depth filtration. §Salt strip 1 M NaCl followed by 2 M. ¶0.22 µm-filtered prior depth filter. #0.22 µm filtered. ##Yunoki* et al. *Unpublished Data.*

*BA: Bioassay (*in vivo *study); BH: Brain homogenate; BSE: Bovine spongiform encephalopathy; CDI: Conformation-dependent immunoassay; CDLs: Caveolae-like domains; dsBH: Detergent treated and sonicated BH; E: Early filtrate; H: High titer of prion on assay of spiked feed stock; IVIG: Intravenous immunoglobulin; L: Low titer of prion on assay of spiked feed stock; La: Late filtrate; M: Middle filtrate; MF: Microsomal fraction; NA: Not applicable; ND: Not determined; PrP: Prion protein; Sc: Scrapie; sMF: Sonicated MF; WB: Western blotting (*in vitro *study).*

summarized study results on the depth filtration of prions in 2004 [31]. However, since the mechanism and consistency of the filtration system is not clear, an evaluation study should be performed using individual manufacturing processes. Thus, it is clear that further knowledge is required regarding the depth filtration systems.

Conclusion

The current status of process evaluation methods for prion removal during manufacturing processes of plasma derivatives was introduced and discussed in this review. Problems to be stressed are:

- The form of pathogenic prions in blood is not clear, which in turn raises questions about the appropriateness of prion materials (spiking materials) used for evaluation studies;
- Preparation methods of prion materials for studies are very important to consider;
- Although some data are already available, the equivalency between model systems and vCJD/CJD must be strengthened.

The current status of the problems and the limitations of measures taken to overcome the problems are described. In addition, the difficulties in establishing conditions for down-scaled experiments are also discussed. More research on spiking materials of model systems for vCJD/CJD is necessary to know whether the currently used materials are appropriate for conducting process evaluation studies. Based on the outcome of such research, it should be carefully judged whether the spiking materials used by model systems are appropriate. At present, ethanol and PEG fractionations, filtration with virus-removal filters, depth filters, protein purification columns and so forth are thought to be effective for prion removal (to some extent).

For virus-removal filters, the partition mechanism is based on size exclusion. The performance of filters in different studies is consistent, and the pore size correlates well with prion removal. However, as aforementioned, variability in the performance is observed depending on the filtration conditions. In contrast to virus-removal filters, depth filters may remove prions more efficiently if process conditions can be optimized. Consequently, virus-removal and depth filters may have great potential for prion removal, although we do not know whether these filter steps (such as depth filters that are adventitiously effective, rather than effective by design) are perfect or not. Therefore, more work is required to establish the filtration conditions that are optimal for prion removal.

Future perspective

In the future, detection methods for pathogenic prions applicable for blood screening are likely to be introduced, and it is expected that the risks of vCJD/CJD transmission through blood will be further clarified. With the introduction of screening tests, it is expected that safety measures for plasma derivatives for prion contamination will be composed of two procedures, screening of source materials and removal during manufacturing processes (the same as for viruses). With progress in the status of pathogenic prions in blood, preparation methods for spiking materials will also likely be optimized. Equivalency between model systems and vCJD/CJD will probably be determined more precisely, although from a safety perspective such studies are challenging. Furthermore, if the usefulness of a quantitative infectivity assay method using cultured cells is confirmed for prion-clearance study, process evaluation could be performed using such an assay. Several techniques to effectively remove prions during the manufacturing process are now under development [46,47]. Once the processes are validated or effective removal has been demonstrated under a variety of process conditions, they may be introduced in actual manufacturing scenarios. The removal ability of processes and the accuracy of process evaluation will be highly improved by combining these new observations and techniques. These improvements will significantly contribute to the safety of plasma derivatives with respect to prion contamination; however, for safety assurance, there is no limit to improvement.

Acknowledgements

The authors thank Andy Bailey, ViruSure GmbH, and Akikazu Sakudo, Osaka University, Japan, for their critical review of this manuscript.

Executive summary

- Incidence of bovine spongiform encephalopathy (BSE) is falling, at least in the UK. Risk of variant Creutzfeldt–Jakob disease transmission derived from BSE-infected bovines is tending to decrease. On the other hand, the risk of human-to-human transmission by blood transfusion persists.

Executive summary

- Sensitive screening methods for the detection of prions in blood are not currently available. Therefore, for plasma derivatives, safety measures against prions are mainly geographical donor deferral and the removal of prions during manufacturing processes.
- Individual manufacturing processes for prion removal are usually evaluated by scaling down the actual manufacturing conditions to laboratory conditions, and using established scrapie strains as model systems. Since the status of the prion protein in blood is not known, the preparation method of prions as spiking materials for these experiments must be considered carefully.
- There are two different procedures to assay prions: *in vitro* study to detect prions by western blotting (WB) and so forth; and *in vivo* study to detect infectious prions by inoculating samples into animals. In some cases for only a slight amount of prion, even when WB demonstrates a negative result, due to the limitation of this technique, for example inappropriate use of antibody, infectivity may be detected in samples. Therefore, for process evaluation, differences between test methods must be considered carefully.
- Depending on process conditions, the prion-removal ability of the process may vary, even if the removal pattern is similar. Thus, the design of the evaluation study is very important. Each manufacturing process must be evaluated independently.
- At present, among the manufacturing processes of plasma derivatives, ethanol fractionation, polyethylene glycol fractionation, column chromatography and filtration with virus-removal filters and depth filters are considered to be effective for prion removal.
- The combination of processes that contribute to prion removal is necessary in order to improve the consistency of the manufacturing processes for prion removal.
- Consequently, the development of new techniques for screening and prion removal, and improvement of process evaluation methods is highly desirable.

Bibliography

Papers of special note have been highlighted as either of interest (•) or of considerable interest (••) to readers.

1. Brown P, Will RG, Bradley R, Asher DM, Detwiler L: Bovine spongiform encephalopathy and variant Creutzfeldt–Jakob disease: background, evolution, and current concerns. *EID* 7(1), 6–16 (2001).
2. Llewelyn CA, Hewitt PE, Knight RS *et al.*: Possible transmission of variant Creutzfeldt–Jakob disease by blood transfusion. *Lancet* 363(9407), 417–421 (2004).
3. Peden AH, Head MW, Ritchie DL, Bell JE, Ironside JW: Preclinical vCJD after blood transfusion in a PRNP codon 129 heterozygous patient. *Lancet* 364(9433), 527–529 (2004).
4. Hopkin M: Fears grow as blood stocks pass on prions undetected. *Nature* 430(7001), 712 (2004).
5. Strengthening of Quality and Safety Assurance of Drugs and Medical Devices Manufactured Using Components of Human Origin as Raw Materials, PFSB Notification No.0209003 dated February 9, 2005, Ministry of Health, Labour and Welfare, Japan (In Japanese).
6. Strengthening of Quality and Safety Assurance of Drugs and Medical Devices Manufactured Using Human- or Bovine-derived Components as Raw Materials, Blood and Blood Products Div. PFSB, Notification No.0725002 dated July 25, 2003 and Evaluation and Licensing Div. PFSB, Notification No.0725001 dated July 25, 2003, Ministry of Health, Labour and Welfare, Japan (In Japanese).
7. Strengthening of Quality and Safety Assurance of Drugs and Medical Devices Manufactured Using Components of Human or Animal Origin as Raw Materials, PMSB Notification No.0414004 dated April 14, 2003, Ministry of Health, Labour and Welfare, Japan (In Japanese).
8. Brown P: Blood infectivity, processing and screening testing transmissible spongiform encephalopathy. *Vox Sang.* 89(2), 63–70 (2005).
   • **Excellent review of information regarding blood product-related prion.**
9. Yao HL, Han J, Gao JM *et al.*: Comparative study of the effects of several chemical and physical treatments on the activity of protease resistance and infectivity of scrapie strain 263K. *J. Vet. Med. B* 52(10), 437–443 (2005).
10. Bauman PA, Lawrence LA, Biesert L *et al.*: Critical factors influencing prion inactivation by sodium hydroxide. *Vox Sang.* 91(1), 34–40 (2006).
11. Kimberlin RH, Walker CA: Characteristics of a short incubation model of scrapie in the golden hamster. *J. Gen. Virol.* 34(2), 295–304 (1977).
12. Kimberlin RH, Walker CA: Evidence that the transmission of one source of scrapie agent to hamsters involves separation of agent strains from a mixture. *J. Gen. Virol.* 39(3), 487–496 (1978).
13. Zlotnik I, Rennie JC: Further observations on the experimental transmission of scrapie from sheep and goats to laboratory mice. *J. Comp. Pathol.* 73, 150–162 (1963).
14. Bruce M, Chree A, McConnell I, Foster J, Pearson G, Fraser H: Transmission of bovine spongiform encephalopathy and scrapie to mice: strain variation and the species barrier. *Phil. Trans. R. Soc. Lond. B* 343(1306), 405–411 (1994).
15. Bruce ME, McConnell I, Fraser H, Dickinson AG: The disease characteristics of different strains of scrapie in Sinc congenic mouse lines: implications for the nature of the agent and host control of pathogenesis. *J. Gen. Virol.* 72(Pt 3), 595–603 (1991).
16. Vey M, Baron H, Weimer T, Gröner A: Purity of spiking agent affects partitioning of prions in plasma protein purification. *Biologicals* 30(3), 187–196 (2002).
17. Brown P, Rohwer RG, Dunstan BC, MacAuley C, Gajdusek DC, Drohan WN: The distribution of infectivity in blood components and plasma derivatives in experimental models of transmissible spongiform encephalopathy. *Transfusion* 38(9), 810–816 (1998).
18. Foster PR, Welch AG, McLean C *et al.*: Studies on the removal of abnormal prion protein by processes used in the manufacture of human plasma products. *Vox Sang.* 78(2), 86–95 (2000).

19. Yunoki M: Evaluation of the ability of planova filtration to eliminate infectious pathogens. *Proceeding of Planova Workshop.* Tokyo, Japan, 1–6 March 2003.
20. Fevrier B, Vilette D, Archer F *et al.*: Cells release prions in association with exosomes. *Proc. Natl Acad. Sci. USA* 101(26), 9683–9688 (2004).
21. Vorberg I, Raines A, Story B, Priola SA: Susceptibility of common fibroblast cell lines to transmissible spongiform encephalopathy agents. *J. Infect. Dis.* 189(3), 431–439 (2004).
22. Lee DC, Stenland CJ, Hartwell RC *et al.*: Monitoring plasma processing steps with a sensitive western blot assay for the detection of the prion protein. *J. Virol. Methods* 84(1), 77–89 (2000).
23. Hartwell RC, Nelson MS, Kislan MM *et al.*: An improved western blot assay to assess the clearance of prion protein from plasma-derived therapeutic proteins. *Biologicals* 34(3), 187–189 (2006).
24. Safar J, Wille H, Itri V *et al.*: Eight prion strains have PrP^{Sc} molecules with different conformations. *Nat. Med.* 4(10), 1157–1165 (1998).
25. Fujita R, Urayama T, Ohmizu A, Yunoki M, Tsuji S, Ikuta K: Efficient detection of PrP^{Sc} (263K) in human plasma. *Biologicals* (2005) (In Press).
26. Fraser H, Dickinson AG: The sequential development of the brain lesions of scrapie in three strains of mice. *J. Comp. Pathol.* 78(3), 301–311 (1968).
27. Kretzschmar HA: Diagnosis of prion diseases. *Clin. Lab. Med.* 23(1), 109–128 (2003).
28. Lee DC, Stenland CJ, Miller JLC *et al.*: A direct relationship between the partitioning of the pathogenic prion protein and transmissible spongiform encephalopathy infectivity during the purification of plasma proteins. *Transfusion* 41(4), 449–455 (2001).
29. Klöhn PC, Stoltze L, Flechsig E, Enari M, Weissmann C: A quantitative, highly sensitive cell-based infectivity assay for mouse scrapie prions. *Proc. Natl Acad. Sci. USA* 100(20), 11666–11671 (2003).
30. Vorberg I, Raines A, Priola SA: Acute formation of protease-resistant prion protein does not always lead to persistent scrapie infection *in vitro*. *J. Biol. Chem.* 279(28), 29218–29225 (2004).
31. Foster PR: Removal of TSE agents from blood products. *Vox Sang.* 87(Suppl. 2), S7–S10 (2004).
32. Cai K, Girman TM, Hotta J *et al.*: Ensuring the biologic safety of plasma-derived therapeutic proteins. *Biodrugs* 19(2), 79–96 (2005).
33. Silveira JR, Raymond GJ, Hughson AG, *et al.*: The most infectious prion protein particles. *Nature* 437(7056), 257–261 (2005).
•• First evidence for size of prion with infectivity.
34. Brown P, Cervenakova L, McShane LM *et al.*: Further studies of blood infectivity in an experimental model of transmissible spongiform encephalopathy, with an explanation of why blood components do not transmit Creutzfeldt–Jakob disease in humans. *Transfusion* 39(11), 1169–1178 (1999).
35. Yunoki M, Urayama T, Nishida M *et al.*: Virus validation study of manufacturing process for antithrombin III. *Iyakuhin Kenkyu* 30(7), 331–341 (1999) (In Japanese).
36. Terpstra FG, Parkkinen J, Tötö H *et al.*: Viral safety of Nanogam, a new 15 nm-filtered liquid immunoglobulin product. *Vox Sang.* 90(1), 21–32 (2006).
37. Stenland CJ, Lee DC, Brown P, Petteway SR Jr, Rubenstein R: Partitioning of human and sheep forms of the pathogenic prion protein during the purification of therapeutic proteins from human plasma. *Transfusion* 42(11), 1497–1500 (2002).
38. Gregori L, Maring JA, MacAuley C *et al.*: Partitioning of TSE infectivity during ethanol fractionation of human plasma. *Biologicals* 32(1), 1–10 (2004).
39. Reichl HE, Foster PR, Welch AG *et al.*: Studies on the removal of a bovine spongiform encephalopathy-derived agent by processes used in the manufacture of human immunoglobulin. *Vox Sang.* 83(2), 137–145 (2002).
40. Trejo SR, Hotta JA, Lebing W *et al.*: Evaluation of virus and prion reduction in a new intravenous immunoglobulin manufacturing process. *Vox Sang.* 84(3), 176–187 (2003).
41. Foster PR, Griffin BD, Bienek C *et al.*: Distribution of a bovine spongiform encephalopathy-derived agent over ion-exchange chromatography used in the preparation of concentrates of fibrinogen and Factor VIII. *Vox Sang.* 86(2), 92–99 (2004).
42. Van Holten RW, Autenrieth S, Boose JA, Hsieh WT, Dolan S: Removal of prion challenge from an immune globulin preparation by use of a size-exclusion filter. *Transfusion* 42(8), 999–1004 (2002).
43. Tateishi J, Kitamoto T, Mohri S *et al.*: Scrapie removal using Planova® virus removal filters. *Biologicals* 29(1), 17–25 (2001).
44. Truchot L, Arnaud T, Bloy C, Perret-Liaudet A: CJD PrP^{Sc} removal by nanofiltration process: application to a therapeutic immunoglobulin solution (Lymphoglobuline®). *Biologicals* 34(3), 227–231 (2006).
45. Van Holten RW, Autenrieth SM: Evaluation of depth filtration to remove prion challenge from an immune globulin preparation. *Vox Sang.* 85(1), 20–24 (2003).
46. Wang K, Johnson A, Obradovic M *et al.*: TSE clearance during plasma products separation process by Gradiflow™. *Biologicals* 33(2), 87–94 (2005).
47. Sowemimo-Coker S, Kascsak R, Kim A *et al.*: Removal of exogenous (spiked) and endogenous prion infectivity from red cells with a new prototype of leukoreduction filter. *Transfusion* 45(12), 1839–1844 (2005).

Websites

101. (In Japanese). www.fsc.go.jp/sonota/qabse1.pdf
102. New case of transfusion-associated vCJD in the United Kingdom (2006). www.eurosurveillance.org/ew/2006/060209.asp2
103. Molesworth AM: Variant Creutzfeldt–Jakob disease in the United Kingdom and elsewhere, situation at the end of 2005. www.eurosurveillance.org/ew/2006/060126.asp4
104. Guideline on the investigation of manufacturing processes for plasma-derived medicinal products with regard to vCJD risk. www.emea.eu.int/pdfs/human/bwp/513603en.pdf
•• Detailed European guideline for the investigation of variant Creutzfeldt–Jacok (vCJD) risk in plasma products.
105. CHMP position statement on Creutzfeldt–Jakob disease and plasma-derived and urine-derived medicinal products. www.emea.eu.int/pdfs/human/press/pos/287902rev1.pdf
106. Note for guidance on the warning on transmissible agents in summary of product characteristics and package leaflets for plasma-derived medicinal products. www.emea.eu.int/pdfs/human/bpwg/056103en.pdf
107. Revised preventive measures to reduce the possible risk of transmission of Creutzfeldt–Jakob disease and variant Creutzfeldt–Jakob disease by blood and blood products. www.fda.gov/cber/gdlns/cjdvcjd.pdf
•• Detailed guideline in the USA for the investigation of vCJD risk in plasma products.

108. Lee D, Lebing W, Stenland C *et al.*: Methods of inactivation of transmissible spongiform encephalopathies as applied to *production chromatography processes*. www.jp.amershambiosciences.com/news letter/downstream/pdf/downstream27.pdf
• Excellent review for the inactivation of prions.
109. Note for guidance on virus validation studies: the design, contribution and interpretation of studies validating the inactivation and removal of viruses. www.emea.eu.int/pdfs/human/bwp/026895 en.pdf
•• Detailed guideline for study design of the virus clearance.

Affiliations

• *Mikihiro Yunoki*
Infectious Pathogen Research Group, Hirakata Research Laboratory, Research & Development Division, Benesis Corporation, 2-25-1, Shodai-ohtani, Hirakata, Osaka 573-1153, Japan
Tel.: +81 72 856 9260;
Fax: +81 72 864 2341;
yunoki.mikihiro@mk.m-pharma.co.jp

• *Takeru Urayama*
Infectious Pathogen Research Group, Hirakata Research Laboratory, Research & Development Division, Benesis Corporation, 2-25-1, Shodai-ohtani, Hirakata, Osaka 573-1153, Japan
Tel.: +81 72 856 9260;
Fax: +81 72 864 2341;
urayama.takeru@mk.m-pharma.co.jp

• *Kazuyoshi Ikuta*
Research Institute for Microbial Diseases, Department of Virology, Osaka University, Suita, Osaka 565-0871, Japan
Tel.: +81 6 6879 8307;
Fax: +81 6 6879 8310;
ikuta@biken.osaka-u.ac.jp

医薬品
医薬部外品　　研究報告　調査報告書
化粧品

識別番号・報告回数			報告日	第一報入手日 2006 年 8 月 9 日	新医薬品等の区分 該当なし	厚生労働省処理欄
一般的名称	①乾燥抗 HBs 人免疫グロブリン ②ポリエチレングリコール処理抗 HBs 人免疫グロブリン		研究報告の公表状況	FDA/CBER/20060808	公表国 アメリカ	
販売名 （企業名）	①ヘブスブリン（ベネシス） ②静注用ヘブスブリン－IH（ベネシス）					

研究報告の概要

FDA/CBER 発行の 2002 年 1 月付" Guidance for Industry: Revised Preventive Measures to Reduce the Possible Risk of Transmission of Creutzfeldt-Jakob Disease (CJD) and Variant Creutzfeldt-Jakob Disease (vCJD) by Blood and Blood Products"の修正ガイダンス案である。勧告内容は以下の通り。
1980 年以降にフランス国内で血液又は血液成分の投与を受けたことのあるドナーは全て永久供血停止とする。但し、この勧告に基づけば供血停止とされるドナーであっても、もし当該ドナーが、非注射剤製造用にのみ血液成分の採取を認める CBER 認可プログラムに関与する場合は、供血を続けるよう推奨する。当該ドナー由来の製品には特別なラベルを付するよう推奨する。
このガイダンス案は、輸血用全血並びに血液成分、及び注射剤の製造に供される血液成分（回収血漿、原料白血球並びに原料血漿を含む）に適用される。

報告企業の意見

FDA/CBER発行の2002年1月付" Guidance for Industry: Revised Preventive Measures to Reduce the Possible Risk of Transmission of Creutzfeldt-Jakob Disease (CJD) and Variant Creutzfeldt-Jakob Disease (vCJD) by Blood and Blood Products"の修正ガイダンス案である。
これまで血漿分画製剤によってvCJDを含むプリオン病が伝播したとの報告はない。しかしながら、万一vCJD感染者の血漿が本剤の原料に混入した場合には、製造工程においてプリオンを低減し得るとの報告があるものの、製剤から伝播する可能性を完全には否定し得ない。そのため、弊社の血漿分画製剤の製造工程におけるTSE感染性低減に関する検証実験を加速し、自社データを早期に取得し、工程評価を行い、必要に応じて工程改善を実施する予定である。

今後の対応

vCJD の疫学情報については、今後も注視することとする。

使用上の注意記載状況・その他参考事項等

代表として静注用ヘブスブリン－IH の記載を示す。
2. 重要な基本的注意
(1)略
1)略
2)現在までに本剤の投与により変異型クロイツフェルト・ヤコブ病（vCJD）等が伝播したとの報告はない。しかしながら、製造工程において異常プリオンを低減し得るとの報告があるものの、理論的な vCJD 等の伝播のリスクを完全には排除できないので、投与の際には患者への説明を十分行い、治療上の必要性を十分検討の上投与すること。

Guidance for Industry

Amendment (Donor Deferral for Transfusion in France Since 1980) to "Guidance for Industry: Revised Preventive Measures to Reduce the Possible Risk of Transmission of Creutzfeldt-Jakob Disease (CJD) and Variant Creutzfeldt-Jakob Disease (vCJD) by Blood and Blood Products"

DRAFT GUIDANCE

This guidance is for comment purposes only.

Submit comments on this draft guidance by the date provided in the *Federal Register* notice announcing the availability of the draft guidance. Submit written comments to the Division of Dockets Management (HFA-305), Food and Drug Administration, 5630 Fishers Lane, rm. 1061, Rockville, MD 20852. Submit electronic comments to http:www.fda.gov/dockets/ecomments. You should identify all comments with the docket number listed in the notice of availability that publishes in the *Federal Register*.

Additional copies of this draft guidance are available from the Office of Communication, Training and Manufacturers Assistance (HFM-40), 1401 Rockville Pike, Suite 200N, Rockville, MD 20852-1448 or by calling 1-800-835-4709 or 301-827-1800, or from the Internet at http://www.fda.gov/cber/guidelines.htm.

For questions on the content of this guidance, contact Dr. Sharyn Orton, Division of Blood Applications at 301-827-3524.

U.S. Department of Health and Human Services
Food and Drug Administration
Center for Biologics Evaluation and Research
August 2006

Table of Contents

Contains Nonbinding Recommendations

Draft – Not for Implementation

Guidance for Industry

Amendment (Donor Deferral for Transfusion in France Since 1980) to "Guidance for Industry: Revised Preventive Measures to Reduce the Possible Risk of Transmission of Creutzfeldt-Jakob Disease (CJD) and Variant Creutzfeldt-Jakob Disease (vCJD) by Blood and Blood Products"

This draft guidance, when finalized, will represent the Food and Drug Administration's (FDA's) current thinking on this topic. It does not create or confer any rights for or on any person and does not operate to bind FDA or the public. You can use an alternative approach if the approach satisfies the requirements of the applicable statutes and regulations. If you want to discuss an alternative approach, contact the appropriate FDA staff. If you cannot identify the appropriate FDA staff, call the appropriate number listed on the title page of this guidance.

I. INTRODUCTION

This draft guidance, which we are issuing as a level I guidance, is intended to amend the "Guidance for Industry: Revised Preventive Measures to Reduce the Possible Risk of Transmission of Creutzfeldt-Jakob Disease (CJD) and Variant Creutzfeldt-Jakob Disease (vCJD) by Blood and Blood Products" (CJD/vCJD guidance), dated January 2002 (Ref. 1), by adding a donor deferral recommendation for donors who have received a transfusion of blood or blood components in France since 1980. After we review comments received on this draft guidance, we will amend the CJD/vCJD guidance by incorporating this donor deferral recommendation, update any outdated information, and reissue the revised CJD/vCJD guidance as a level II guidance document for immediate implementation.

This draft guidance applies to Whole Blood and blood components intended for transfusion, and blood components intended for use in further manufacturing into injectable products, including recovered plasma, Source Leukocytes and Source Plasma. Special provisions apply to donors of blood components intended solely for manufacturing of non-injectable products (see section III). Within this document, "donors" refers to donors of Whole Blood and blood components and "you" refers to blood collecting establishments.

FDA's guidance documents, including this guidance, do not establish legally enforceable responsibilities. Instead, guidances describe FDA's current thinking on a topic and should be viewed only as recommendations, unless specific regulatory or statutory requirements are cited. The use of the word *should* in FDA's guidances means that something is suggested or recommended, but not required.

II. BACKGROUND

Since the publication of the CJD/vCJD guidance, we have learned of additional information warranting revision to the guidance to address a possible increased risk of vCJD transmission from individuals who have been transfused in France since 1980. This revision is based on (1) the likelihood of exposure to the Bovine Spongiform Encephalopathy (BSE) agent in that country and (2) the recent documentation of three presumptive cases of transfusion-transmitted vCJD infection in the United Kingdom (U.K). As of August 1, 2005, 14 definite or probable cases of vCJD have been reported in France (Ref. 2).

Available data suggest that large amounts of U.K. beef exported to France during the peak years of the U.K. BSE epidemic constituted a substantial source of exposure in France to the BSE agent. An estimated 60% of U.K. bovine carcasses were exported to France (Ref. 3) accounting for approximately 6% of French consumption of beef products (Ref. 4). It is believed that the first recognized vCJD cases in France were infected by consuming imported U.K. beef because: 1) none of the individuals had lived in the U.K.; 2) the indigenous French BSE epidemic is relatively small and more recent than that in the U.K.; and 3) travels to the U.K. accounted for only 2% of the French total exposure to the BSE agent (Ref 3).

There have been a total of three presumptive cases of transfusion-transmitted vCJD, and all have been in the U.K. The first presumptive transfusion-transmitted case of vCJD by red blood cells was reported to the U.K. Parliament on December 17, 2003 (Ref. 5). A second presumptive case was reported in the U.K. in 2004 (Ref. 6). A third presumptive case was publicly announced by authorities in the U.K. in 2006 (Ref. 7).

On February 8, 2005, the Transmissible Spongiform Encephalopathies Advisory Committee (TSEAC) discussed the available data and recommendations for deferral of U.S. donors transfused since 1980 in France and in other European countries. The TSEAC voted for deferral of blood donors who have received a transfusion of blood or blood components in France since 1980 but against deferral of Source Plasma donors with that same history. The TSEAC did not support deferral of blood donors or Source Plasma donors with history of transfusion in other European countries since 1980 (Ref. 8).

The incubation period for classical CJD may be as long as 38.5 years. Accumulating evidence suggests that the asymptomatic incubation periods of vCJD may be very long as well (sometimes exceeding 12 years from the time of exposure to the BSE agent), and blood collected as long as three years before otherwise healthy blood donors showed any sign of illness is presumed to have transmitted vCJD infection to recipients (Refs. 5 and 6). While the risk of dietary exposure to the BSE agent in France, as in the U.K. and other European countries, has almost certainly decreased in recent years thanks to successful efforts to control the BSE epidemic in cattle and to protect food from contamination with the BSE agent, an unknown but possibly significant number of blood donors might have already been infected in France during the peak years of the BSE outbreak in Europe. These considerations led FDA, consistent with the recommendations of the TSEAC, to conclude that it would be a prudent

preventive measure to indefinitely defer blood donors who have received transfusions of blood or blood components in France since 1980. Laboratory studies using model TSE agents have demonstrated that TSE infectivity may be reduced by certain plasma fractionation manufacturing steps (Ref. 9). While experimental studies are reassuring, not all products have been thoroughly studied. In addition, it remains uncertain whether the models accurately reflect the form of infectivity in blood, which has not been characterized. Therefore, as an added safeguard and prudent preventive measure, we also recommend that Source Plasma donors who have received a transfusion of blood or blood components in France since 1980 be indefinitely deferred. However, we believe that blood components collected solely for manufacturing into non-injectable products (e.g., materials used in in vitro diagnostic test kits) need not be deferred. We will continue to monitor the BSE epidemic and re-evaluate the necessity of deferring donors transfused in other European countries.

III. RECOMMENDATIONS

You should indefinitely defer all donors who have received a transfusion of blood or blood components in France since 1980.

NOTE: Donors who are otherwise deferred based upon this recommendation should continue to donate if they are participating in a CBER-approved program that allows collection of blood components solely for use in manufacturing of non-injectable products. We recommend special labeling for products obtained from such donors (see section VII.A of the CJD/vCJD guidance).

All other recommendations from the CJD/vCJD guidance remain unchanged.

IV. IMPLEMENTATION

We recommend that you implement this donor deferral recommendation within six months of the date that we finalize this draft guidance amendment. This draft guidance amendment will be finalized by reissuing the CJD/vCJD guidance inclusive of the amended language.

V. REFERENCES

1. FDA "Guidance for Industry: Revised Preventive Measures to Reduce the Possible Risk of Transmission of Creutzfeldt-Jakob Disease (CJD) and Variant Creutzfeldt-Jakob Disease (vCJD) by Blood and Blood Products," January 2002; http://www.fda.gov/cber/gdlns/cjdvcjd.htm.
2. http://www.invs.sante.fr/publications/mcj/donnees_mcj.html
3. Chadeau-Hyam, M. and A. Alperovitch, "Risk of Variant Creutzfeldt-Jakob Disease in France," *International Journal of Epidemiology*, 34(1):46-52, 2005.
4. Supervie, V. and D. Costagliola, "The Unrecognised French BSE Epidemic," *Veterinary Research*, 35(3):349-62, 2004.
5. Llewelyn, C.A., P.E. Hewitt, R.S. Knight, et al., "Possible Transmission of Variant Creutzfeldt-Jakob disease by Blood Transfusion," *Lancet*, 363(9407):417-21, 2004.
6. Peden A.H., M.W. Head, D.L. Ritchie, et al., "Preclinical vCJD After Blood Transfusion in a PRNP Codon 129 Heterozygous Patient," *Lancet*, 364(9433):527-9, 2004.
7. http://www.eurosurveillance.org/ew/2006/060209.asp
8. http://www.fda.gov/ohrms/dockets/ac/05/transcripts/2005-4088t1.doc
9. Foster, P.R., A.G. Welch, C. McLean, et al., "Studies on the Removal of Abnormal Prion Protein by Processes Used in the Manufacture of Human Plasma Products," *Vox Sanguinis*, 78:86-95, 2000.

医薬品
医薬部外品　研究報告　調査報告書
化粧品

別紙 3-3

識別番号・報告回数		回	報告日 年　月　日	第一報入手日 2006 年 2 月 28 日	新医薬品等の区分 該当なし	総合機構処理欄
一般的名称			研究報告の公表状況	Clinical implications of emerging pathogens in haemophilia: the variant Creutzfeldt-Jakob disease experience Golan, G. Haemophilia 12. (Suppl. 1), 16 - 20 (2006)	公表国 英国	
販売名（企業名）						

研究報告の概要	使用上の注意記載状況・その他参考事項等
本文献は英国の血友病の実地臨床における変異型クロイツフェルト・ヤコブ病(vCJD)の影響を報告している。1980年代，血漿由来製剤による治療で，血友病患者団体がHIVとC型肝炎ウイルス感染の危険に直面するという厳しい教訓を得た。このため，1997年に英国血友病センターの医師団は，血友病患者に対する最適な治療法は遺伝子組換え凝固因子であると述べた。1996年の，英国で最初のvCJD症例報告の直後に，輸血を介してvCJD感染の恐れがあるという懸念が生じた。1997年，vCJDは英国においてのみ確認され，多くの血友病患者が英国由来の血漿因子濃縮製剤の投与を受けていたため，遺伝子組換え凝固因子による治療を受けられない患者には，非ヨーロッパ諸国，好ましくは米国で処理が行われた血漿因子濃縮製剤を用いるべきである，という追加勧告がなされた。後に，1996-97年に英国で供血された血漿因子濃縮製剤のいくつかのバッチが実際にvCJDを引き起こす病原因子で汚染されていたことが明らかになった。この結果，2005年4月現在，A型及びB型血友病患者全員に遺伝子組換え凝固因子が投与されている。輸血を介してvCJDに感染した最初の患者が2003年12月に死亡後，ヒト間での感染リスクが減少するよう計画された法案により，1980年から2001年の間に英国で供血されたヒト血漿由来の治療を受けた全血友病患者の取扱いが決定した。例えば，これらの患者が中枢神経系に関わる手術を受けた際，使用された手術器具は全て廃棄されなければならない。したがって，筆者は血友病患者にはリスクの最も低い治療法のみを適用することを推奨している。	BYL-2006-0220-3

報告企業の意見	今後の対応
弊社の第 VIII 因子製剤は遺伝子組換え製剤である。本剤の培養培地にはヒト血漿由来成分が使用されているが，弊社の血漿分画製剤の製造工程は，4log を上回るプリオンを除去することが確認されている。	現時点で新たな安全対策上の措置を講じる必要は無いと考える。引き続き関連情報の収集に努める。

Haemophilia (2006), 12, (Suppl. 1), 16–20

Clinical implications of emerging pathogens in haemophilia: the variant Creutzfeldt–Jakob disease experience

G. DOLAN
Department of Haematology, University Hospital, Queen's Medical Centre, Nottingham, UK

Summary. The impact of variant Creutzfeldt–Jakob disease (vCJD) on the clinical practice of haemophilia in the UK is coloured by the haemophilia community's experience of hepatitis C virus and human immunodeficiency virus (HIV) transmission via plasma-derived therapies in the 1980s, when the delay in recognizing and acting on the potential risks cost many patients their lives and left others to manage another chronic disease. This crisis prompted organisations such as the United Kingdom Haemophilia Centre Doctors' Organisation to advocate for the introduction of haemophilia therapies that would not be susceptible to contamination with blood-borne pathogens. After the identification of vCJD in 1996, a number of public health measures were taken in response to a government-sponsored vCJD risk assessment, and following reports of transfusion-transmission of vCJD, additional guidelines have been developed to prevent person-to-person transmission, some of which may impact the quality and availability of medical and surgical care. Variant CJD has had a significant negative effect on the UK haemophilia community, shaking patient confidence in the therapies they have received over the last 21 years, affecting the quality of care and creating the risk of stigmatizing the community as it was in the 1980s. As with HIV and vCJD, emerging blood-borne infectious agents will likely affect blood and blood-derived therapies well before we become aware of its presence. As a result, only therapies with the lowest level of risk should be used for care of patients with haemophilia.

Keywords: haemophilia, pathogen, variant Creutzfeldt–Jakob disease

Introduction

This article will review the impact of variant Creutzfeldt–Jakob disease (vCJD) on the clinical practice of haemophilia in the UK, with particular attention to how haemophilia treater and patient organizations have responded to this concern. The haemophilia community's response to vCJD is best understood in the context of the significant morbidity and mortality caused by the transfusion-transmitted hepatitis C virus (HCV) and human immunodeficiency virus (HIV) infections contracted in the 1980s. Given the delayed recognition of the risk that HIV and HCV posed to patients with haemophilia, the subsequent lack of rapid response and the many missed opportunities to protect patients from contaminated plasma-derived therapies, it is understandable that many patients with haemophilia and their caregivers are now very alert to the potential implications of emerging pathogens such as vCJD. This is especially true for those patients who still rely on plasma-derived therapies and transfusions.

Correspondence: Gerry Dolan, MB, ChB, FRCP, FRC Path, Department of Haematology, University Hospital, Queen's Medical Centre, Nottingham NG7 2UH, UK.
Tel.: + 44 0 115 970 9187; fax: + 44 0 115 970 9786;
e-mail: gerry.dolan@nottingham.ac.uk

UKHCDO therapeutic guidelines

The United Kingdom Haemophilia Centre Doctors' Organisation (UKHCDO) was established in 1968 by doctors treating patients with bleeding disorders who sought to improve care, conduct research into the disorders and facilitate healthcare planning. The UKHCDO and the patient organization the Haemophilia Society had, for many years, argued for the introduction of recombinant therapies. This view was reflected in the UKHCDO haemophilia treatment guidelines, published in 1997, which stated that recombinant factor concentrates were the treatment of choice for patients with haemophilia [1]. The guidelines further stated that recombinant factor concentrates were the safest with respect to reducing the risk of transfusion-transmitted infection. At the

time the UKHCDO guidelines were released, the general consensus among haemophilia treaters was that the plasma therapies used in the UK had a relatively low risk for transmission of hepatitis or HIV, but because they could transmit other infectious agents, such as parvovirus B19 and hepatitis A, [2,3] they might in theory be the route of infection for new or altered agents.

The UKHCDO guidelines were accepted by most treaters but not by the majority of healthcare commissioners. In particular, the future risk of infection by emerging pathogens through plasma therapy was not accepted. Approximately 6 months later, the potential threat of vCJD to the haemophilia community emerged.

Shortly after vCJD was first described in the UK in 1996, concerns were raised that it could be transmitted through blood transfusion and blood therapies [4]. As a result, the UKHCDO convened a meeting with experts on prion diseases, including members of the National CJD Surveillance Unit and the Spongiform Encephalopathy Advisory Committee (SEAC), both of which were formed in 1990. The National CJD Surveillance Unit is sponsored by the Department of Health (DOH) and the Scottish Executive Health Department; SEAC is sponsored jointly by the Department for Environment, Food and Rural Affairs, the DOH and the Food Standards Agency (FSA). The purpose of the meeting was to determine, by means of a thorough review of all available evidence, if there were any measures available to effectively reduce the risk to patients with haemophilia of contracting vCJD and other prion-based diseases.

At the time, in 1997, vCJD had only been identified in Great Britain. Limited research indicated that this was a new disease with a long incubation period [5]. Relatively little epidemiological data were available, but evidence from some animal studies indicated that there existed the possibility of transfusion-transmitted vCJD infections. Further, it was surmised that many vCJD-infected, yet asymptomatic, individuals were continuing to donate blood that would be used in the processing of factor VIII and factor IX therapies. At that time, many patients with haemophilia in the UK were treated with UK-sourced plasma factor concentrates.

Based on the 1997 meeting of the UKHCDO, SEAC and the National CJD Surveillance Unit, several recommendations emerged [4]:

1 Healthcare providers should reduce the risk of vCJD transmission by using plasma factor concentrates sourced in other countries.

2 Recombinant factor concentrates should remain the treatment of choice for patients with haemophilia.

3 Plasma-derived concentrates processed with non-European plasma, preferably from the US, should be provided for those patients for whom recombinant factor concentrates were not made available.

As a consequence of these recommendations, the two main UK fractionators of plasma, Bio Products Laboratory and the Scottish National Blood Transfusion Service, were obligated to stop processing factor concentrate therapies. In the meantime, the UK imported plasma from the US for processing factor VIII and factor IX. This ban on utilization of UK-derived plasma resulted in long delays in resuming the processing of factors and interrupted the supply of other niche therapies such as factor VII and factor XI.

Patients and providers respond

Prior to 1997, many patients with haemophilia and their physicians held the view that UK-sourced plasma therapies were safer than any alternative and there had been a relatively slow uptake of recombinant therapies. With the introduction of these policies recommending the use of non-UK-sourced plasma, however, patient confidence was undermined and the pressure increased on government and healthcare commissioners to make recombinant therapies more widely available.

Against a background of increasing concern about the possible risk of vCJD, England's Department of Health agreed that recombinant therapies should be made available to all children with haemophilia [6]. In other health departments, in Scotland, Wales and Northern Ireland, they took the recommendations one step further and introduced recombinant therapies for all patients. But in England, the most populous country in the UK, adults continued to be prescribed and use plasma therapies, although derived from plasma imported from the USA.

Variant CJD: a potential new threat to factor concentrate safety

In 2000, Bio Products Laboratory notified the UKHCDO about the identification of batches of factor concentrates that had been prepared in 1996 and 1997 and used before 1998. It was determined that these concentrates were prepared from plasma pools that included plasma from a donor who had

subsequently developed vCJD. Since then there have been further notifications of batches of factor concentrates prepared from plasma from donors who were later diagnosed with vCJD. Table 1 enumerates all the batches of therapies distributed and subsequently identified as being potentially infected with vCJD, as of September 2004 [7]. These therapies were produced by either Bio Products Laboratory or Protein Fractionation Centre and, in most circumstances, many patients were treated with these therapies before notification had been given.

At the time there was no clear evidence that vCJD could be transmitted by blood products. There was no test to identify potentially asymptomatic but infected donors, and there was no treatment to offer patients for reassurance or for further assessment. Because vCJD has a long incubation period, clinical examination was of little or no use. With these facts in mind, healthcare providers and policy makers were faced with the decision of what, or even if, to tell their patients.

Response to possible risk of transfusion-transmitted vCJD

In 2004, the decision was made to inform all patients about the possible risk of transfusion transmitted vCJD, irrespective of whether they had received concentrates or not from the implicated batches. Patients were given three choices: they could come into their healthcare providers' offices and discuss the information in person; they could choose to be fully informed by letter; or they could refuse to be informed in any way. Many patients chose the third option. Patients who chose to be educated about the potential risks were given information disclosing that they might be infected with vCJD. Given that the majority of patients were not able to have access to recombinant therapies, this situation caused considerable concern.

For the UKHCDO, responding to the potential infection of haemophilia patients created a huge administrative burden. There was an urgent need to review all records, to contact all patients possibly infected and to give each of them the option to review all information then known about vCJD. Added to the administrative burden were government-mandated timelines as to when the patients needed to be informed.

Table 1. Batches of 'implicated' UK plasma therapies [7].

Factor VIII	16*
Factor IX	8*
Antithrombin	1
Immunoglobulin G	11
Albumin 4.5%	28
Albumin 20%	21
Factor VIII with albumin excipient	76
Intramuscular immunoglobulin	12

*Indicates widely distributed throughout the UK.

The threat of vCJD among members of the haemophilia community increased the political pressure for more widespread use of recombinant coagulation factor concentrates in the UK. And as a result, as of April 2005, all patients with haemophilia A and B have been offered recombinant factor concentrates.

Risk of vCJD from implicated plasma-derived concentrates

One of the questions that remain unanswered today is what risk do the recipients of plasma concentrates exposed to vCJD pose to others? This issue came to the forefront in December 2003 when the Health Secretary informed the UK Parliament of the first death probably related to transfusion-transmitted vCJD. This case was later confirmed as being related to vCJD [8,9].

The Department of Health established the CJD Incidents Panel, an expert committee sub group of the Advisory Committee on Dangerous Pathogens Working Group on Transmissible Spongiform Encephalopathies, in 2000 in order to help the medical community handle cases such as this. The mandate of this committee is to review the available literature, establish a formal risk assessment of infectivity of blood and blood therapies and formulate guidelines for response by the medical community. The CJD Incidents Panel advises hospitals, trusts and public health teams throughout the UK on how to manage incidents involving possible transmission of CJD between patients.

Based on a risk assessment commissioned by the DOH in 2003, the CJD Incidents Panel attempted to identify patients who had received at least one dose of a plasma therapy, which the committee judged to increase the risk of vCJD exposure by more than 1% over background. Therapies that were considered the highest risk were factor VIII, factor IX and antithrombin. The administration of just one vial, or 500 units, was considered enough to put patients in a high-risk category. Medium risk therapies included intravenous immunoglobulin G and albumin 4.5% administered in large doses. Low-risk therapies were defined as albumin 20%, intramuscular immunoglobulin and factor VIII with excipient albumin administered in extremely large doses [10].

In refining the risk assessment, the question emerged: which of the 'at risk' patients need be treated with precaution: those with known exposure to contaminated or potentially contaminated batches of plasma concentrates, or any patient treated with plasma-derived concentrate in the period from 1980 to 2001? Because the possibility existed that, over time, additional donors might be identified as having vCJD, it was decided to treat all haemophilia patients who had used therapies from UK-derived plasma in this 21-year-period with measures designed to reduce the risk of human-to-human transmission [11].

Measures to prevent human-to-human vCJD transmission

Following the 2001 release of a DOH-sponsored summary of the risks of vCJD transmission via surgical implements [12], the Advisory Committee on Dangerous Pathogens and the Spongiform Encephalopathy Advisory Committee published a set of guidelines in 2003 for the precautionary management of potentially-infected patients, both healthy and deceased, in order to minimise the risks of transmission to other patients and healthcare staff [13]. These guidelines were a significantly expanded version of recommendations that were released in 1998 but kept under review until a number of uncertainties were better understood, including the routes of infection, threshold infectious dose, potential for inactivating the agent and the quantity of people who might be incubating the disease.

The detailed guidelines recommend measures for laboratory containment and control, infection control of CJD and related disorders in a healthcare setting, decontamination and waste disposal and quarantining of surgical instruments, among others. For example, when patients who used UK-sourced plasma-based therapies in the years 1980–2001 undergo any surgery involving high-risk tissues, such as the central nervous system or the lymphatic system, the surgical instruments used must be subsequently destroyed [14].

Some general precautions included using single-use instruments wherever possible; performing all procedures in a controlled environment, such as an operating theatre; performing the procedure after all others; involving the minimum number of healthcare personnel; and using liquid-repellent operating gowns over plastic aprons, as well as goggles or full-face visors [15].

More controversially, the guidelines stipulated that if these patients have an endoscopic procedure of the gastrointestinal tract or the olfactory mucosa, the instruments used in those procedures also must be quarantined, i.e. not used again or destroyed [15]. The quarantine or destruction of surgical instruments has, of course, financial consequences: the quarantine of an endoscope is estimated to cost approximately £30 000 per instrument per year. Endoscopy services are in high demand, and quarantining an endoscope, or destroying it after every use, is not a reasonable or cost-effective policy for any healthcare institution. In the risk-assessment guidelines, it was suggested that capsule wireless endoscopes be used instead, but expertise in capsule endoscopy is limited, so the issue has yet to be fully resolved.

Potential stigmatization

One of the negative outcomes of the distribution of the guidelines of the CJD Incidents Panel was that persons with haemophilia became identified as presenting a risk of infection to others. In some medical centres, reluctance to performing invasive procedures became an issue in all but serious cases.

Despite assertions that these precautions should not compromise care for patients with haemophilia, the potential exists that these patients will be stigmatized again, as they were early in the HIV crisis, and that their normal medical and surgical care may be interrupted.

Scope of the problem

Cases of vCJD have also been reported outside the UK. In France, for example, 14 cases of vCJD have been reported, with three identified in persons who donated blood over a 10-year-period. Again, most of the donations have been used to make factor VIII, von Willebrand factor, and other plasma therapies. In response, the French have recalled all plasma-derived therapies, where possible, and all patients have been informed.

To further complicate matters, it is known that the French fractionators have exported concentrates to other countries, such as Belgium. And in the UK, Bio Products Laboratory also exported factor concentrate to other countries. At this point in time, there are no clear guidelines on how to manage potential risk in these situations.

Another concern involves haemophilia patients who visited the UK: unknown numbers of visitors were treated with UK-sourced factor concentrates during the crucial 21-year-period. Because records on the treatment of visitors to the UK are not readily

available, it is very difficult to identify or advise those patients.

Conclusion

The phenomenon of emerging vCJD is yet another warning against the complacent assumption that plasma-derived therapies can be made completely safe. Variant CJD has had a significant negative effect on the haemophilia community in the UK, shaking patient confidence in the therapies they have received over the last 21 years, affecting the quality of current and future medical and surgical care and creating the risk of stigmatizing the community as it was in the 1980s, at the beginning of the HIV crisis.

Our awareness of vCJD is not even a decade old. Much about the disease is still unknown, including the best means for preclinical detection and effective inactivation. But given its long incubation period, it's possible that the impact of vCJD on patients with haemophilia may be significant.

As described elsewhere in this supplement, the barriers to the emergence of pathogenic agents, both air- and blood-borne, continue to diminish. And as with HIV and vCJD, the next emerging blood-borne infectious agent will likely affect blood and blood-derived therapies well before we become aware of its presence. It is because of these reasons that only the therapies with the lowest level of risk should be used for care of patients with haemophilia.

References

1 United Kingdom Haemophilia Centre Doctors' Organisation. Guidelines on therapeutic products to treat haemophilia and other hereditary coagulation disorders. *Haemophilia* 1997; 3: 63–77.

2 Soucie JM, Siwak EB, Hooper WC, Evatt BL, Hollinger FB and the Universal Data Collection Project Working Group. Human parvovirus B19 in young male patients with hemophilia A: associations with treatment product exposure and joint range-of-motion limitation. *Transfusion* 2004; 44: 1179–85.

3 Schneider B, Becker M, Hans-Hermann B, Eis-Hubinger AM. Contamination of coagulation factor concentrates with human parvovirus B19 genotype 1 and 2. *Thromb Haemost* 2004; 92: 838–45.

4 Ludlam CA, on behalf of the executive Committee of the UKHCDO. New-variant Creutzfeldt–Jakob disease and treatment of haemophilia. *Lancet* 1997; 350: 1704.

5 Will RG. Variant Creutzfeldt–Jakob disease. *Acta Neurobiol Exp* 2002; 62: 167–73.

6 National Health Service. *Provision of Recombinant Factor VIII for New Patients and Children Under the Age of 16*. Health Service Circular. HSC 1998/033. 17 March 1998. Published online at http://www.dh.gov.uk/assetRoot/04/01/16/95/04011695.pdf. Accessed September 2005.

7 Health Protection Agency. *vCJD and Plasma Products – Tables of vCJD Implicated Batch Numbers: To Those Responsible for Tracing vCJD Implicated Plasma Product Batches in the UK*. 7 September 2004. Published online at http://www.wfh.org/2/docs/Safety_Supply/Recall_BPL_Sept2004.pdf. Accessed September 2005.

8 Llewellyn CA, Hewitt PE, Knight RSG *et al.* Possible transmission of variant Creutzfeldt–Jakob disease by blood transfusion. *Lancet* 2004; 363: 417–21.

9 Peden AH, Head MW, Ritchie DL, Bell JE, Ironside JW. Preclinical vCJD after blood transfusion in a PRNP codon 129 heterozygous patient. *Lancet* 2004; 364: 527–9.

10 Det Norske Veritas for Department of Health. *Risk Assessment of Exposure to vCJD Infectivity in Blood and Blood Products*. February 2003, 1–30. Published online at http://www.dnv.co.uk/Binaries/vCJD_Update_Report_tcm23-74414.pdf. Accessed September 2005.

11 Health Protection Agency. *vCJD and Plasma Products – Clinical Information*. 7 September 2004. Published online at http://www.hpa.org.uk/infections/topics_az/cjd/Clinical.pdf. Accessed September 2005.

12 Department of Health, Economics and Operational Research Division (EOR4). *Risk Assessment for Transmission of vCJD via Surgical Instruments: a Modelling Approach and Numerical Scenarios*. February 2001. Skipton House, London. Published online at http://www.dh.gov.uk/assetRoot/04/07/53/88/04075388.pdf. Accessed September 2005.

13 Advisory Committee on Dangerous Pathogens and the Spongiform Encephalopathy Advisory Committee. Foreword. In: *Transmissible Spongiform Encephalopathy Agents: Safe Working and the Prevention of Infection*, 2003. Available at http://www.advisorybodies.doh.gov.uk/acdp/tseguidance/. Accessed September 2005.

14 Advisory Committee on Dangerous Pathogens and the Spongiform Encephalopathy Advisory Committee. Annex E: Quarantining of surgical instruments. In: *Transmissible Spongiform Encephalopathy Agents: Safe Working and the Prevention of Infection*, 2003. Published online at http://www.advisorybodies.doh.gov.uk/acdp/tseguidance/tseguidance_annexe.pdf. Accessed September 2005.

15 Advisory Committee on Dangerous Pathogens and the Spongiform Encephalopathy Advisory Committee. Part 4: Infection control of CJD and related disorders in the healthcare setting. In: *Transmissible Spongiform Encephalopathy Agents: Safe Working and the Prevention of Infection*, 2003. Published online at http://www.advisorybodies.doh.gov.uk/acdp/tseguidance/tseguidancepart4.pdf. Accessed September 2005.

医薬品
医薬部外品　研究報告　調査報告書
化粧品

別紙 3-4

識別番号・報告回数		回	報告日 年　月　日	第一報入手日 2006 年 2 月 28 日	新医薬品等の区分 該当なし	総合機構処理欄
一般的名称			研究報告の公表状況	Implications of emerging pathogens in the management of haemophilia Haemophilia 12, (Suppl. 1), 26 -28 (2006)	公表国 米国	
販売名（企業名）						

研究報告の概要

英国保健当局と医療関係者間の以下2点に関する議論の要旨である。
i) 現在の血友病患者が直面している新規の，血液媒介病原体による潜在的感染の危険性
ii) 過去25年間にこの分野で判明した教訓
2番目の議論に関して，英国の血友病患者にとってエイズまたは変異型クロイツフェルトヤコブ病(vCJD)の危険性が大きな脅威であった。これまで，血液凝固第VIII因子，又は第IX因子療法を受けている患者のvCJD症例報告はなかった。しかし，正式にこの可能性を否定するには，恐らく以前より時間がかかるであろう。また，白血球除去や血漿分画といった技術改善は感染症のリスクを減少させる際に僅かに効力を示すのみであった。
最近では，遺伝子組換え製剤による治療は完全にウイルス感染リスクをなくすわけではない，ということが明らかになっている。特に，現在の不活性化法に耐性のあるパルボウイルスB19のような非エンベロープウイルスとなるとなおさらである。西ナイルウイルスのような血液を介して感染する新規病原体は予想以上に早く出現する，と医師はみている。そのため彼らは最高水準の安全管理を推奨し，新規病原体のスクリーニングテストが可能となれば即時，血友病患者に対して積極的に行い，適切な手段と規制を実施することを提案している。

使用上の注意記載状況・その他参考事項等

BYL-2006-0220-4

報告企業の意見	今後の対応
弊社の第 VIII 因子製剤は遺伝子組換え製剤であるが，本剤の培養培地にはヒト血漿由来成分が使用されている。しかしながら，弊社製剤中に残存しうる血漿由来成分は，プール血漿においてNAT を実施するとともに，その製造工程でウイルス不活化・除去工程を実施し安全性を確保している。	現時点で新たな安全対策上の措置を講じる必要は無いと考える。引き続き関連情報の収集に努める。

(26)

Haemophilia (2006), 12, (Suppl. 1), 26–28

DISCUSSION SESSION

Implications of Emerging Pathogens in the Management of Haemophilia

Discussion Session

1. Is there any evidence that haemophilia patients in the UK have been infected with variant Creutzfeldt–Jakob disease (vCJD) via therapies made from contaminated blood donations? Phrased differently, are there good data to support the decision in the UK to phase out the use of recombinant factor VIII (rFVIII) therapies processed with plasma additives, and are the surgical precautions in treating haemophilia patients necessary?

DOLAN: Initial discussions surrounding these issues were definitely controversial, and we in the medical community were not sure how far we needed to go in trying to protect patients. But the recommendations and surgical measures were devised after very detailed consultation with experts who knew far more about prion disease than we did.

Certain decisions, such as ceasing use of UK plasma-derived therapies, were difficult for both patients and their providers. But the subsequent events, in particular the later evidence that there have been at least two probable cases of transfusion-transmitted variant CJD, seem to justify that early stance by not just the UK but other countries as well.

2. Do you think that the fact that vCJD has not been identified in any patient receiving plasma derivatives worldwide since 1980 suggests that the risk of vCJD is minimal or non-existent from these therapies?

IRONSIDE: First of all, let's be quite clear about why 1980 has become a benchmark. The date 1980 was chosen simply because that was thought to be the earliest date at which human exposure to bovine spongiform encephalopathy (BSE) in the UK was likely to have occurred. Overall, human exposure to BSE probably would be very low in the early 1980s and highest in the late 1980s and early 1990s. It is also important to remember that we are dealing with a primary disease transmission with an incubation period of approximately 15 years on average. So, we may have to wait a few more years before we can be certain about the absolute risk of contracting vCJD.

I would be very cautious about relaxing policies and guidelines at present because, as we all understand, there are other emerging infectious agents – identified and unidentified – that are cause for concern in addition to the vCJD-causing prion.

3. Do you know of any vCJD transmissions by plasma-derived FVIII/FIX therapies?

IRONSIDE: At present, no. There is no evidence that vCJD has occurred or infection has been transmitted by these therapies. Although, as I stated earlier, this may be due to the fact that we are dealing with an agent that has a long incubation period. The level of infectivity in plasma therapies may be lower or variable. But it is too soon to exclude that possibility.

The United Kingdom Haemophilia Centre Doctors' Organisation, along with several patient groups, is engaged in enhanced surveillance of the haemophilia population. We are looking for evidence of vCJD – even of subclinical infection – in patients who died or who have a lymphoid tissue biopsy for whatever reason.

4. What is the likely impact of the UK experience with vCJD in the United States and what might those treatment implications be?

DOLAN: Reported cases of BSE in the United States are very few. And if the number of cases remains at this low level, or even disappears altogether, then perhaps US practitioners and policy makers won't be obligated to take the more sweeping measures that we did in the UK. However, as a general concept, we must all remember that emerging pathogens can affect transfusion therapy. So, based on the UK experience, if healthcare providers have an opportunity to minimize risk to patients, then it is a prudent course of direction that should be considered seriously and likely taken.

5. Are there data that leukodepletion of blood will decrease the risk of transmitting vCJD? If not, what is the rationale?

IRONSIDE: This is a very interesting question because the UK has been using leukodepletion as one of its main strategies for risk reduction in terms of blood transfusion. The data from experimental

studies do indicate that although leukodepletion will reduce infectivity, it will not remove it entirely.

Because leukodepletion does not remove all infectivity, there have been a number of other approaches that utilize additional filters that might bind more specifically to any free prion protein in the plasma and thus, further reduce the risk.

6. Please describe the results of experiments in which blood was spiked with vCJD concentrate to determine whether prions could be removed.

IRONSIDE: Results of a spiking experiment were published using blood containing a range of prions, including both sporadic and variant CJD prions. The study looked at the effect of plasma fractionation in removing the prions. And indeed, fractionation did seem to have a positive effect.

However, there are a number of concerns about these spiking experiments because they involve inoculating brain homogenate into blood and using that as the spike. Essentially, it is infected brain tissue, which is very unphysiological. Therefore, it is unlikely to replicate the form of infectivity found in blood-endogenous infection, where it is probably free in plasma and not aggregated as it would be in brain. So, while the spiking experiments do provide some reassuring information, a number of questions persist as to just how valid the spiking method is.

7. What about the results of the study in which 11% of patients who received recombinant therapy only were seropositive for parvovirus B19 antibodies soon after start of treatment? Aren't recombinant therapies totally free of any virus transmission risk?

TAPPER: As has been stated, the non-lipid-encased viruses are obviously much more difficult to inactivate. So if you ask, do the current technologies inactivate all pathogens, the answer is clearly no, they do not.

Parvovirus is one of the classic markers for these types of viruses. In children, parvovirus is relatively benign, but older people tend to get sick from it. Parvovirus can be viewed as a marker for pathogens that are either difficult to inactivate or that simply have not been fully described as yet. There are many viruses that fall into this latter category. For example, where did severe acute respiratory syndrome come from? Where did the coronavirus come from? It is clearly a novel virus that probably made a cross-species jump. You could say very much the same thing about human immunodeficiency virus when it was first described in industrialized countries in the 1980s, but clearly, phylogenetically, it had been present in Africa for at least 50 years prior to that time.

Factors such as the vastly increased ability of populations to travel, the issues surrounding land encroachment and the disruptions of the natural barriers between humans and humans and between humans and animals are clearly going to continue. And within that context, you can anticipate that new pathogens will continue to emerge, at least some of which, like West Nile virus, will be transmissible via blood.

PIPE: The medical community is not particularly concerned with parvovirus, but we're looking at it as a marker because it is one of the non-lipid-enveloped viruses for which we can actually screen. At this point in time, the theoretical concern would involve early seroconversions among patients who have depended solely on recombinant therapies. We would need to ask: is there the potential for another infectious agent – which either has or has not emerged yet, or that we don't have a test for – to become a threat to these patients?

What it comes down to is an issue of vigilance, and I think it is encouraging to see that when testing is available, such as prion screening, we are actively looking for patients who have the protein. Another encouraging example involves West Nile virus. It was only a very short period of time from its appearance to actually having an effective screening tool; this rapid response illustrates that the scientific world can respond quickly to address these kinds of issues.

8. What is the justification of continuing to use a therapy that is processed with bovine plasma protein?

PIPE: In a single clinic, I might talk to a patient with von Willebrand disease and a patient with another rare coagulation deficiency, both of whom would rely on plasma derivatives. With these patients I discuss the continued vigilance and screening that have resulted in the safety of these therapies thus far. I think it is important to inform them that there are ongoing concerns with respect to emerging pathogens, but also that as we learn more about potentially infective agents, we establish policies that will go a long way toward preventing another crisis in which emerging pathogens contaminate blood-derived therapies.

Alternatively, I will have a conversation with a family member or patient with either haemophilia A or haemophilia B and discuss with them the availability of newer therapies that are not processed with human or animal protein additives. The conversation with the patient with von Willebrand disease is very different than the one with the haemophilia patient: one is a conversation of reassurance, and the other a conversation of striving to be proactive, to help these

patients and their caregivers consider new therapies that may reduce the risk of infection with disease-causing agents.

Our history with haemophilia patients is interesting. In 1992, we switched all of our paediatric patients on FVIII to recombinant therapies. Then, in 1998 when recombinant FIX was available, we switched all of our patients from plasma-derived FIX to recombinant. That therapy had reduced recovery time in paediatric patients, and as a result, many patients had to use up to twice the amount of factor units that they would have had they remained on plasma-derived therapies. There is also the increased cost associated with the therapy.

The decision to switch patients to recombinant therapies was not based on any evidence of a known infectious agent being transmitted by plasma derivatives. Yet if you look at the data from the US Centers for Disease Control and Prevention on the adoption of recombinant therapies in paediatric patients, and indeed for adult patients around the US, it is quite remarkable how enthusiastically patients and clinicians have embraced recombinant technology.

For some patients, unfortunately, choice is not an option. There are patients in some areas of the US who do not even have access to recombinants. So, for these patients we must rely on the 20 years of safety that we have enjoyed with plasma derivatives. This relative safety should not lull us into a mode of complacency where we ignore emerging pathogens such as vCJD.

医薬品
医薬部外品　研究報告　調査報告書
化粧品

識別番号・報告回数		回	報告日 年　月　日	第一報入手日 2006 年 2 月 28 日	新医薬品等の区分 該当なし	総合機構処理欄
一般的名称			研究報告の公表状況	Variant Creutzfeldt-Jakob disease: risk of transmission by blood transfusion and blood therapies Ironside, J.W. Haemophilia 12, (Suppl. 1), 8 - 15 (2006)	公表国 英国	
販売名（企業名）						

研究報告の概要

このレビュー記事は最新のvCJDに関する知見と英国での罹患率の要旨である。vCJDは感染性海綿状脳症(TSE)である。TSEの原因物質は立体的に構造変化した宿主正常タンパク質(プリオンタンパク質，PrP^{C})であり，異常で毒性を持つタンパク質(PrP^{Sc}：異常プリオンタンパク質)を作り出し中枢神経系に蓄積される。これまで，vCJDの臨床症例はすべてプリオンタンパク遺伝子のコドン129がメチオニン同型の人で発現していた。ウシ海綿状脳症(BSE)の蔓延は1986年の英国に始まり，何千頭ものウシに影響を及ぼした。その一方，ヒトで初のvCJDはその10年後に報告され，汚染牛肉の消費が原因と思われた。その後すぐに，血液を介したプリオン感染が齧歯類で実験的に証明され，同様の血液感染ルートによるヒト間での感染の可能性という懸念が生じた。後に，無症候のドナーから供血を受けた後にvCJDを発現した2症例が実際に報告された。興味深いことに，1症例目の患者はvCJDが原因で死亡し，そのコドン129はメチオニン同型であった。一方，2症例目の患者はvCJDの徴候はなく無関係の状態で死亡し，そのコドン129は異型であった。
ヒトにおけるvCJDの潜伏期間は不明であり，血液中のPrP^{Sc}を検出するスクリーニングテストがないため，英国においてvCJDの無症候段階(供血者になり得る)にある感染者数を知るのは現時点では不可能である。逆に，vCJDの臨床症状は発症しないが，無症候キャリアーとして感染させる可能性のある人々の存在も浮き彫りになった。
衛生局がリスク評価を行った結果，第VIII因子，第IX因子及び抗トロンビン使用患者はvCJDに感染するリスクが最も高くなった。これらの使用患者をこれまで以上に保護するために，異常なPrP^{Sc}の検出に特定する高感度の血液検査を開発し，同様に潜在的感染者の疫学的調査を頻繁に行うことが不可欠である。
2005年10月の時点で，世界中でvCJD184症例が確認されていると報告された：英国158症例，フランス15症例，その他EU諸国7症例，日本1症例，米国1症例，カナダ1症例，サウジアラビア1症例。カナダ，日本，米国の感染者とアイルランドの感染者1名は英国に在住した履歴があった。そのため，日本政府は1980～1996年の間に英国へ渡航したドナーからの献血を禁止した。

使用上の注意記載状況・その他参考事項等

BYL-2006-0220-2

報告企業の意見	今後の対応
弊社血漿分画製剤に使用している血漿は，vCJD のリスクが低い米国で採漿されており，また，現在までに血漿分画製剤による vCJD 感染症例は報告されていないことから，弊社の血漿分画製剤におけるリスクは依然低いと考える。	現時点で新たな安全対策上の措置を講じる必要は無いと考える。引き続き関連情報の収集に努める。

Haemophilia (2006), 12, (Suppl. 1), 8–15

Variant Creutzfeldt–Jakob disease: risk of transmission by blood transfusion and blood therapies

J. W. IRONSIDE
University of Edinburgh, National CJD Surveillance Unit, Western General Hospital, Edinburgh, UK

Summary. In the last decade, a new variant of the human prion disease Creutzfeldt–Jakob disease (now known as variant CJD or vCJD) was identified and causally linked to dietary exposure to bovine spongiform encephalopathy (BSE) during the 1980s and early 1990s. Preliminary studies in animal models suggest that prions can be transmitted by blood. Based on two recent reports of iatrogenic vCJD transmission by blood transfusion in humans, a Department of Health-sponsored risk assessment warned that recipients of plasma therapies are now at risk of contracting vCJD from potentially infected donors. It is believed that all the population may be susceptible to vCJD infection, although clinical cases have so far occurred only in methionine homozygotes at codon 129 in the human prion protein gene. A non-invasive blood-based diagnostic assay is urgently needed. Because the incubation period may be upwards of 40 years and there is no reliable screening test, it is currently unknown how many people may be in an asymptomatic phase of vCJD infection in the UK. However, there remains a distinct possibility that some infected patients may never develop clinical symptoms but will remain asymptomatic carriers who can potentially transmit the disease to other individuals. Therefore, screening of infectious individuals will be a critical component for individuals who rely on blood transfusions and/or blood therapies. In the absence of screening tests or effective therapies to treat this disease, a formidable worldwide public health challenge lies ahead to prevent new infections, accurately assess infection rates and treat infected patients.

Keywords: blood transfusion, factor replacement, haemophilia, prion, transmission, variant Creutzfeldt–Jakob disease

Introduction

Variant Creutzfeldt–Jakob disease (vCJD) is a recently identified member of the transmissible spongiform encephalopathies (TSE) or prion diseases [1,2]. These disorders are fatal neurodegenerative conditions occurring in humans and other mammals, the best known examples in non-human species being bovine spongiform encephalopathy (BSE) in cattle, scrapie in sheep and chronic wasting disease in deer and elk [3]. Prion diseases are transmissible under both experimental and natural conditions. For many years, the nature of the transmissible agent was the subject of intense debate, and in 1982 the prion hypothesis was proposed by Prusiner [4]. This postulated that the transmissible agent was composed entirely of a modified host protein (prion protein) that was partially resistant to proteolytic degradation, without a nucleic acid component.

The normal form of the prion protein (PrP^{C}) is expressed in many cells and tissues in the body, but is present at highest levels in neurones within the central nervous system [3]. The precise function of PrP^{C} is uncertain, but it has a short half life and is readily degraded by proteolytic enzymes [5]. An abnormal isoform of PrP (PrP^{Sc}) accumulates in the central nervous system in prion diseases. PrP^{Sc} has an identical amino acid sequence to PrP^{C}, but a different conformation, with an increased beta-sheet content that is associated with infectivity and neurotoxicity [3]. This abnormal conformation also confers a relative resistance to degradation by proteolytic enzymes. The precise cellular mechanisms that result in this conformational change, and their locations, have not yet been fully determined.

Correspondence: James W. Ironside, FRCPath University of Edinburgh, National CJD Surveillance Unit, Western General Hospital, Edinburgh, EH4 2XU UK.
Tel.: 44 131 537 3109; fax: 44 131 537 3056;
e-mail: james.ironside@ed.ac.uk

The BSE epidemic in the UK

In 1987, a novel progressive neurological condition in cattle was reported in the UK [6]. The new disease was named bovine spongiform encephalopathy (BSE, or 'mad cow' disease) because of its similarity to other prion diseases by pathology and immunohistochemistry. By the early 1990s thousands of cattle were diagnosed with BSE and millions were incinerated to prevent the disease from spreading [7,8]. However, BSE has still not been fully eradicated in the UK. The BSE epidemic in the UK has been attributed to TSE-infected feeds made of meat and bone meal prepared from rendered sheep offal [9]. With the prohibition of specific feeding practices and specified offals, however, the number of reported cases declined to fewer than 500 by 2003 in UK (Fig. 1) [7,8].

Since the UK continued to export cattle offals after 1986, the BSE agent spread to over 20 European countries, as well as to Japan, Russia, Canada, Israel and the USA. Thus, the exportation of contaminated animal feed from the UK to many other countries across the world resulted not only in the spread of BSE but potentially widespread human exposure to BSE-positive animals through the consumption of BSE-contaminated meat products [10]. Public health concerns about the safety of meat products around the world since the BSE epidemic two decades ago have not diminished. On 24, June 2005, the US Department of Agriculture confirmed BSE in a cow that had conflicting screening test results the previous year. Fortunately, no part of the animal had entered the human or animal food supply; however, this case heightened the awareness of the need for better testing in this country and ongoing surveillance [8,11].

Table 1. Classification of human prion diseases [12].

Class	Diseases
Idiopathic	Sporadic Creutzfeldt–Jakob disease
	Sporadic fatal insomnia
Familial	Familial Creutzfeldt–Jakob disease
	Gerstmann–Sträussler–Scheinker syndrome
	Fatal familial insomnia
Acquired	
Human origin	Kuru, iatrogenic Creutzfeldt–Jakob disease
Bovine origin	Variant Creutzfeldt–Jakob disease

Classification of human prion diseases

Human prion diseases are categorized into three distinct groups that reflect their different origin and range: idiopathic, inherited and acquired [2] (Table 1). The commonest of the idiopathic disorders is sporadic CJD (sCJD). Sporadic CJD is distributed worldwide and is the most common of all human prion diseases, accounting for around 85% of all cases [13]. It is associated with a highly aggressive clinical course with a mean duration of illness of approximately 4.5 months. Sporadic CJD occurs most frequently in middle-aged or elderly individuals and appears to be triggered by a somatic mutation of the prion gene, or by a spontaneous conformational change of the host prion protein from its normal cellular form (PrP^{C}) to its abnormal and pathogenic form (PrP^{Sc}) [3,14].

Inherited (familial) forms of prion diseases comprise up to 15% of all cases and are strongly linked to a series of pathogenic mutations and insertions in the prion protein gene [15,16]. The clinical course of these TSEs is characterized by a slow degeneration of the central nervous system, resulting in dementia, ataxia, motor difficulties and death. The inherited human prion diseases comprise three main groups of

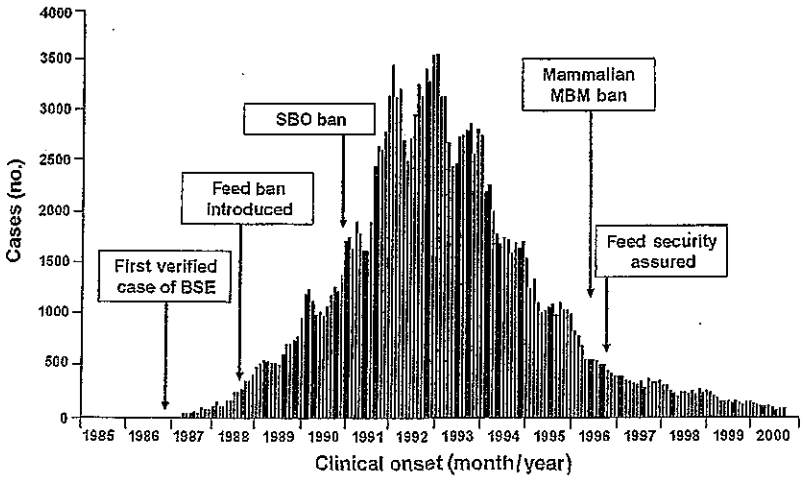


Fig. 1. Bovine spongiform encephalopathy epidemic in the UK [7].

disorders, each with a characteristic clinical and pathological phenotype: familial CJD, the Gerstmann–Sträussler–Scheinker syndrome and fatal familial insomnia [16]. All occur as autosomal dominant disorders [15].

The third group of human prion diseases, the acquired disorders, comprise <1% of all cases and are characterized by exposure to infectivity in brain or nervous system tissue either through human-to-human contact via contaminated neurosurgical instruments, tissue grafts or extracts (iatrogenic CJD) [17], or via the consumption of contaminated bovine meat products (vCJD). Experimental transmission studies have shown that the transmissible agent in vCJD has identical properties to the BSE agent, confirming the link between these 2 disorders [18,19].

Variant CJD was first described in the UK in 1996, but has now been identified in 10 other countries. Variant CJD tends to affect young adults, with a mean age of approximately 29 years (age range 12–74 years at disease onset) [1]. Interestingly, this corresponds with the general age group at which people become blood donors. The duration of the clinical illness is longer (mean duration of 13 months) than that of sCJD, and is characterized by psychiatric features and sensory symptoms at onset, followed by ataxia, myoclonus and other movement disorders; rapidly progressive dementia is very uncommon in this disease [1]. Thus, sCJD and vCJD are distinct disorders that are characterized by different geographical distributions, durations of illness, ages of onset and clinical course, and, most importantly, the causal association of vCJD with BSE.

Transmission of prion diseases by blood

While the transmission of prion infectivity through blood in rodent models of scrapie is well established, recent reports have also found evidence of infectivity in the blood of a rodent model of vCJD and in sheep experimentally infected with BSE [20,21]. These findings have raised questions over the potential transmission of vCJD by blood or blood components. Therefore, concern over safeguarding the blood supply has been gradually mounting given the potentially large number of asymptomatic carriers of vCJD who may unknowingly donate blood. This threat to the blood supply poses a unique challenge to public health officials and raises concerns for patients – especially individuals with haemophilia and other bleeding disorders – who routinely rely on the blood supply and blood therapies. Retrospective studies of haemophilia patients who died from other diseases, including HIV, have not identified any cases of sCJD that were missed or misdiagnosed, either in the UK or in the USA [22,23]. However, although epidemiological studies of sCJD have found no convincing evidence of its transmission by blood [24], the different pathogenesis of vCJD does not allow reassurance to be taken from these studies focusing on sCJD.

Genetic susceptibility to vCJD

Progress in the understanding of human prion diseases was accelerated following the identification of the PrP gene on the short arm of chromosome 20. The identification of pathogenic mutations and insertions in the PrP gene provided evidence to support the prion hypothesis, as familial prion disorders are both genetic and transmissible. Furthermore, it is now recognized that a polymorphism at codon 129 in the human PrP gene may influence susceptibility to prion disease.

Three genetic subgroups have been identified at codon 129 of the PrP gene: methionine homozygous (M/M), valine homozygous (V/V) and heterozygous (M/V). All clinical cases of vCJD have so far occurred in individuals with the methionine homozygous genotype [25,26]. This finding is important because only around 40% of the total human population are methionine homozygotes; approximately 10% are valine homozygotes and 50% are heterozygotes [27,28,29] (Table 2). However, among sCJD cases, only 65% are methionine homozygotes. Thus the methionine homozygous genotype is more susceptible to developing both sporadic and vCJD.

Diagnostic assays for vCJD

One of the largest issues that confront clinicians trying to manage this disease is the absence of a diagnostic screening test for vCJD. Confirmation of a clinical diagnosis of vCJD requires neuropathological examination of the brain following autopsy, with demonstration of the characteristic type 2B isoform of PrP^{Sc} in the brain and lymphoid tissues [25].

Table 2. PRNP codon 129 genotype frequencies [29].

	Genotype		
	M/M	M/V	V/V
Normal population	37%	51%	12%
Sporadic CJD	65%	17%	18%
Variant CJD	100%	–	–

CJD, Creutzfeldt–Jakob disease; M/M, methionine homozygous; M/V, valine heterozygous; V/V, valine homozygous.

Therefore, diagnostic assays are urgently needed for vCJD that are blood based and do not require an invasive brain or tonsil biopsy [30].

A major challenge to the development of such a test is that prions are devoid of nucleic acid, unlike bacteria or viruses, making rapid polymerase chain reaction-based diagnostics non-viable. In addition, as prions are modified cellular proteins and not foreign, there is an absence of a measurable host immune response; hence, an enzyme-linked immunoadsorbent assay (ELISA) diagnostic test is not feasible. The best diagnostic marker for prion diseases is the presence of the disease-associated isoform of the prion protein, PrP^{Sc} [30]. This is generally detected by western blot assay in the brain and in lymphoid tissues in vCJD [31], but attempts to detect PrP^{Sc} in blood from patients with vCJD have so far been unsuccessful, probably because of limitations in the sensitivity of this assay [32]. However, a conformation-dependent immunoassay was recently described that measures both the protease-resistant and protease-sensitive forms of PrP^{Sc} [33] and appears to be far more sensitive than western blot assays. Whether this method will be applicable to blood samples remains to be seen. Another technique that has recently been developed for enhanced detection of PrP^{Sc} is the cyclical amplification method [34]. This relies on a repeated series of incubation with normal PrP and subsequent cycles of sonication, and has recently detected PrP^{Sc} in blood from a rodent model of TSE [35].

Probable pattern of tissue infectivity in vCJD

In the UK, it is presumed that most of the adult population was exposed to the BSE agent through the ingestion of contaminated meat products in the late 1980s and early 1990s. However, because the incubation period of BSE in humans is unknown (incubation periods of 40 years or longer have been documented for other human TSE) [17], and because of the lack of a reliable screening test, it is currently unknown how many people may be in an asymptomatic phase of vCJD infection in UK.

In contrast to sCJD, vCJD infectivity is more widely distributed outside the CNS, and can readily be found in the peripheral nervous system and lymphoid tissues (tonsil, spleen, lymph node and gut) [31]. The levels of infectivity in these tissues are lower than in the CNS, but they still represent possible sources of person-to-person spread of infectivity (Fig. 2) [36]. As the asymptomatic phase of infection in vCJD may last for at least several years, infected individuals may represent a potential source

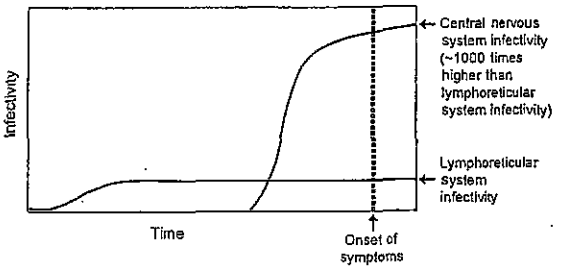


Fig. 2. Probable pattern of tissue infectivity in variant Creutzfeldt–Jakob disease [36].

of secondary spread of vCJD to others via contaminated surgical instruments (such as tonsillectomy instruments) or by blood transfusion.

Variant CJD prevalence study in UK

To estimate the number of individuals in the UK who are asymptomatic for vCJD and who could potentially contribute to the iatrogenic spread of the disease, a retrospective study of lymphoid tissues was recently performed using immunohistochemistry for prion protein in surgically removed tonsillectomy and appendectomy specimens. Researchers reported three positive samples out of 12 674 tested, or an estimated prevalence of 237 vCJD cases per million in the UK (CI 95%) [37,38].

These findings indicate a far higher prevalence than clinical cases would predict, suggesting that additional cases of vCJD are likely to emerge in the UK. Furthermore, they emphasize the importance of preventive measures already instituted by the UK Department of Health to reduce the potential spread of vCJD through blood therapies. These findings also point to the urgent need for large-scale screening of lymphoreticular tissue samples to determine with greater precision the incidence of vCJD infection in the asymptomatic UK population [38].

However, there remains a distinct possibility that some infected patients may never develop clinical symptoms but will remain asymptomatic carriers who can potentially transmit the disease to other individuals. Therefore, screening of infectious individuals will be a critical component for individuals who rely on blood transfusions and/or blood therapies.

Transmission of vCJD infectivity via blood transfusion in humans

Two cases of probable iatrogenic vCJD transmission through blood transfusion have been reported. The first case was a 69-year-old male who presented with

clinical symptoms typical of vCJD in 2002, 6.5 years after receiving one unit of non-leucodepleted packed red blood cells [39]. This patient died 1 year later. Sequencing of the prion protein gene revealed that he was methionine homozygous at codon 129 of the prion protein gene. The asymptomatic donor developed symptoms 3.5 years after donation and subsequently died.

The second case was an elderly female patient who was a known recipient of a blood transfusion from an asymptomatic donor who later developed vCJD [40]. The female patient died of an unrelated illness and without any vCJD clinical symptoms. Because of her known exposure, a medicolegal autopsy was performed. Abnormal prion protein was detected in the spleen and lymph nodes; however, PrP^{Sc} was not detected in the CNS and there were no other significant abnormalities in the CNS. Interestingly, this patient was heterozygous (M/V) at codon 129 in the prion protein gene.

Because that was the first identified case of vCJD infection occurring in the heterozygous subgroup [40], this case raises many important issues regarding the disease, including whether this genotype may have influenced either its incubation period or distribution of infectivity in this patient. These findings underscore the importance of developing effective screening tools and techniques to identify blood donors who may be asymptomatic. In addition, they highlight the need to ascertain whether all vCJD/BSE infections result in clinical disease or whether a subclinical carrier state may occur.

Epidemiological considerations

In the absence of a transfusion-transmitted infection, one statistical analysis has estimated that the probability of acquiring vCJD is approximately 1 in 15,000 to 1 in 30,000 [39]. Therefore, while dietary exposure can never entirely be ruled out, in the aforementioned cases, the infections were far more likely associated with vCJD-contaminated blood transfusions.

To examine a probable link between transfusion and vCJD infection, a review of blood transfusion policies in the UK and a risk assessment on the implications for plasma therapy recipients was commissioned by the Department of Health [41]. The commissioned research concluded that the infectivity concentrations in blood were likely to be highest in the buffy coat fraction, followed by those in plasma and whole blood (Table 3). Moreover, the report stated that levels of the infectious agent present in a full unit of blood would probably be sufficient to cause infection in recipients [41]. The Department of Health's Health Protection Agency also evaluated the risk of different plasma products in an attempt to determine which were most likely to carry the greatest degree of vCJD infectivity. Recipients of factor VIII, factor IX and antithrombin were estimated to have the highest risks: administration of even a single one-vial dose of these products was determined to be sufficient to cause transmission of the disease [42]. Intravenous immunoglobulin (IVIG) and large doses of albumin were concluded to be of medium risk, and anti-D and IVIG were determined to be of low-risk of infectivity.

Table 3. Selected infectivity of blood components [41].

	Volume (mL unit^{-1})	Infectivity (ID_{50}/unit)	Infectivity concentration (ID_{50}/unit)
Whole blood	450	900	2.0
Plasma	225	480	2.1
Filtered plasma	225	480	2.1
Red cells	212	219	1.0
Buffy coat	14	201	14.9

The risk of contracting vCJD from plasma therapies

As recipients of plasma therapies appear to possess the highest risk of contracting vCJD, it is theoretically possible that many patients with bleeding disorders in the UK have already been exposed to the agent responsible for vCJD. Patient groups and the UK Haemophilia Centre Doctors' Organisation believed that the Health Protection Agency's CJD Incidents Panel should recommend that all patients with bleeding disorders in the UK who were treated with UK-source pooled factor concentrates between 1980 and 2001 be considered at potential additional risk for public health purposes [42].

The risk of contracting vCJD has implications for the overall safety of the worldwide blood supply. To address this concern, various measures have been taken to protect the blood supply in the UK, including the sourcing of plasma from the United States (Table 4). Future efforts to minimize the risk of prion contamination of the blood supply might include improved filtration steps to more effectively remove this pathogen.

Variant CJD worldwide as of October 2005

As of October of 2005, 184 confirmed cases of vCJD have been reported worldwide. Individual countries include: UK (158), France (15), Ireland (3), Italy (1),

Table 4. Measures taken to reduce the risk of variant Creutzfeldt–Jakob disease (vCJD) transmission via blood and blood therapies in the UK.

Date	Measure
1997	Withdrawal and recall of any blood components, plasma therapies or tissues obtained from any individual who develops vCJD
1998	Importation of plasma from the USA for fractionation
1998–1999	Leucodepletion of all blood used for transfusion
2002	Importation of fresh plasma from the USA for patients born on or after 1, January 1996
2004	Blood donation is not accepted from people who have received a blood transfusion in the UK since 1980, or who are unsure of this
2005	Donors of blood to patients who have subsequently developed vCJD are advised that they may be at 'increased risk' of vCJD and should not continue to donate blood
Today	Promotion of appropriate use of blood and alternatives in NHS
The future?	Use of 'prion filters'?

USA (1), Canada (1), Saudi Arabia (1), Japan (1), the Netherlands (1), Spain (1) and Portugal (1). The individuals in the USA, Canada and Japan who contracted vCJD and one person in Ireland had all lived in the UK; therefore, these four cases are considered as UK infections.

Japan confirmed its first case of vCJD in 2005. This patient had briefly visited the UK in the late 1980s, fell ill in 2001 and died in 2004. While BSE has been identified in 15 Japanese cattle, officials contend that the patient most likely contracted the disease while in the UK [43]. Because the patient is believed to have visited the UK for less than a month, the Japanese government has changed its blood donation policy to ban donations from anyone who visited UK for a day or more between 1980 and 1996. Previously its policy had been to accept blood donors who had visited the UK for up to 1 month [44].

The fact that cases of vCJD have been reported in many different countries suggest that the disease has spread from the UK to other continents. Although the number of deaths per annum of vCJD in the UK has steadily declined from 28 in the year 2000 to only two by the middle of 2005, the onset of new cases has gradually risen to nine in 2004 from five in 2003 [45]. These data suggest that the disease may become endemic at a low level in the UK population.

Research priorities for vCJD

There are four immediate research priorities. First, to reduce the potential spread of vCJD, there is an urgent need for development of a new screening assay that is applicable to blood and is both highly specific and sensitive. Second, enhanced epidemiological surveillance of potentially infected donors should be broadened to encompass all age groups in the UK. Third, improved methods of decontamination of surgical and laboratory instruments must be developed and implemented across the country to reduce further iatrogenic infections. Finally, progress in the treatment and prophylaxis of vCJD is desperately needed.

Conclusions

In the last decade, a variant of CJD has emerged in many countries that has been causally linked to dietary exposure to BSE during the 1980s and early 1990s. Preliminary studies in animal models suggest that prions, including the BSE agent, can be transmitted by blood. Based on two recent reports of iatrogenic vCJD transmission by blood transfusion in humans, a UK DOH-sponsored risk assessment warned that recipients of plasma therapies are now at risk of contracting vCJD from potentially infected donors. In the absence of screening tests or effective therapies to treat this disease, a formidable worldwide public health challenge lies ahead to prevent new infections, accurately assess infection rates and treat infected patients.

Acknowledgements

The National CJD Surveillance Unit in the UK is supported by the Department of Health and the Scottish Executive Health Department. I am grateful to clinicians and pathologists in the UK for their co-operation in the investigation and diagnosis of all forms of CJD.

References

1 Will RG, Zeidler M, Stewart GE *et al.* Diagnosis of new variant Creutzfeldt–Jakob disease. *Ann Neurol* 2000; 47: 575–82.
2 Ironside JW. Creutzfeldt–Jakob disease. *Brain Pathol* 1996; 6: 379–88.
3 Prusiner SB. Prions. *Proc Nat Acad Sci USA* 1998; 95: 13363–83.
4 Prusiner SB. Novel proteinaceous infectious particles cause scrapie. *Science* 1982; 216: 136–44.
5 Borchelt DR, Scott M, Taraboulos A, Stahl N, Prusiner SB. Scrapie and cellular prion proteins differ in their kinetics of syntheses and topology in cultured cells. *J Cell Biol* 1990; 110: 743–52.
6 Wells GA, Scott AC, Johnson CT *et al.* A novel progressive spongiform encephalopathy in cattle. *Vet Rec* 1987; 121: 419–20.

7 Department for Environment, Food and Rural Affairs. BSE Statistics. 2005, Published online at http://www.defra.gov.uk/animalh/bse/statistics/incidence.html. Accessed September 2005.

8 Priola S, Vorberg I. Molecular aspects of disease pathogenesis in the transmissible spongiform encephalopathies. *Methods Mol Biol* 2004; 268: 517–40.

9 Prusiner SB. Biology and genetics of prion diseases. *Ann Rev Microbiol* 1994; 48: 655–86.

10 Roma AA, Prayson RA. Bovine spongiform encephalopathy and variant Creutzfeldt–Jakob disease: how safe is eating beef? *Cleve Clin J Med* 2005; 72: 185–6, 189–90, 192–4.

11 Centers for Disease Control and Prevention. BSE (bovine spongiform encephalopathy, or mad cow disease), second BSE-positive cow identified in the United States. Revised June 27, 2005. Published online at http://www.cdc.gov/ncidod/dvrd/bse/bse_cow_june_2005.htm. Accessed September 2005.

12 Ironside JW, Ritchie DL, Head MW. Phenotypic variability in human prion diseases. *Neuropathol Appl Neurobiol* 2005; 31: 565–79.

13 Ward HJT, Everington D, Croes EA *et al.* Sporadic Creutzfeldt–Jakob disease and surgery: a case-control study using community controls. *Neurology* 2002; 59: 543–8.

14 Trevitt CR, Singh PN. Variant Creutzfeldt–Jakob disease: pathology, epidemiology, and public health implications. *Am J Clin Nutr* 2003; 78: 651–66.

15 Goldfarb LG, Brown P, Cervenakova L, Gajdusek DC. Molecular genetic studies of Creutzfeldt–Jakob disease. *Mol Neurobiol* 1994; 8: 89–97.

16 Kovács GS, Trabattoni G, Hainfellner JA, Ironside JW, Knight RSG, Budka H. Mutations of the prion protein gene: phenotypic spectrum. *J Neurol* 2002; 249: 1567–82.

17 Brown P, Preece M, Brandel J-P *et al.* Iatrogenic Creutzfeldt–Jakob disease at the millennium. *Neurology* 2000; 55: 1075–81.

18 Bruce ME, Will RG, Ironside JW *et al.* Transmissions to mice indicate that 'new variant' CJD is caused by the BSE agent. *Nature* 1997; 389: 498–501.

19 Scott MR, Will R, Ironside J *et al.* Compelling transgenetic evidence for transmission of bovine spongiform encephalopathy prions to humans. *Proc Natl Acad Sci USA* 1999; 96: 15137–42.

20 Houston F, Foster JD, Chong A, Hunter N, Bostock CJ. Transmission of BSE by blood transfusion in sheep. *Lancet* 2000; 356: 999–1000.

21 Cervenakova L, Yakoleva O, McKenzie C *et al.* Similar levels of infectivity in the blood of mice infected with human-derived vCJD and GSS strains of transmissible spongiform encephalopathy. *Transfusion* 2003; 43: 1687–94.

22 Lee CA, Ironside JW, Bell JE *et al.* Retrospective neuropathological review of prion disease in UK haemophilic patients. *Thromb Haemost* 1998; 80: 909–11.

23 Evatt BL. Prions and haemophilia: assessment of risk. *Haemophilia* 1998; 4: 628–33.

24 Esmonde TF, Will RG, Slattery JM *et al.* Creutzfeldt–Jakob disease and blood transfusion. *Lancet* 1993; 341: 205–7.

25 Ironside JW, Head MW, Bell JE, McCardle L, Will RG. Laboratory diagnosis of variant Creutzfeldt–Jakob disease. *Histopathology* 2000; 37: 1–9.

26 Clark P, Ghani A. Projections of the future course of the primary vCJD epidemic in the UK: inclusion of subclinical infection and the possibility of wider genetic susceptibility. *J R Soc Interface* 2005; 2: 19–31.

27 Owen F, Poulter M, Collinge J, Crow TJ. Codon 129 changes in the prion protein gene in Caucasians. *Am J Hum Genet* 1990; 46: 1215–6.

28 Collinge J, Palmer MS, Dryden AJ. Genetic predisposition to iatrogenic Creutzfeldt–Jakob disease. *Lancet* 1991; 337: 1441–2.

29 Alperovitch A, Zerr I, Pocchiari E *et al.* Codon 129 prion protein genotype and sporadic Creutzfeldt–Jakob disease. *Lancet* 1999; 353: 1673–4.

30 MacGregor I. Prion protein and developments in its detection. *Transfus Med* 2001; 11: 3–14.

31 Ironside JW, McCardle L, Horsburgh A, Lim Z, Head MW. Pathological diagnosis of variant Creutzfeldt–Jakob disease. *APMIS* 2002; 110: 79–87.

32 Wadsworth JD, Joiner S, Hill AF *et al.* Tissue distribution of protease resistant prion protein in variant Creutzfeldt–Jakob disease using a highly sensitive immunoblotting assay. *Lancet* 2001; 358: 171–80.

33 Safar JG, Geschwind MD, Deering C *et al.* Diagnosis of human prion disease. *Proc Natl Acad Sci USA* 2005; 102: 3501–6.

34 Saborio GP, Permanne B, Soto C. Sensitive detection of pathological prion protein by cyclical amplification of protein misfolding. *Nature* 2001; 411: 810–3.

35 Castilla J, Saa P, Soto C. Detection of prions in blood. *Nat Med* 2005; 11: 982–5.

36 CJD Incidents Panel. *Management of Possible Exposure to CJD through Medical Procedures: Framework Document.* UK, 2005. Published online at http://www.hpa.org.uk/infections/topics_az/cjd/framework_Aug%202005.pdf. Accessed September 2005.

37 Ironside JW, Hilton DA, Ghani A *et al.* Retrospective study of prion-protein accumulation in tonsil and appendix tissues. *Lancet* 2000; 355: 1693–4.

38 Hilton DA, Ghani AC, Conyers L *et al.* Prevalence of lymphoreticular prion protein accumulation in UK tissue samples. *J Pathol* 2004; 203: 733–9.

39 Llewelyn CA, Hewitt PE, Knight RSG *et al.* Possible transmission of variant Creutzfeldt–Jakob disease by blood transfusion. *Lancet* 2004; 363: 417–21.

40 Peden AH, Head MW, Ritchie DL, Bell JE, Ironside JW. Preclinical vCJD after blood transfusion in a PRNP codon 129 heterozygous patient. *Lancet* 2004; 364: 527–9.

41 Det Norske Veritas for Department of Health. *Risk Assessment of vCJD Infectivity in Blood.* Appendix II.

Infectivity of Blood. February 2003. Published online at http://www.dnv.com/binaries/AppII_tcm4-74416.pdf. Accessed September 2005.

42 Health Protection Agency. *Variant Creutzfeldt–Jakob Disease (vCJD) and Plasma Products*. Clinical information. 2004. Published online at http://hpa.org.uk/infections/topics_az/cjd/Clinical.pdf. Accessed September 2005.

43 MacKenzie D. First human case of mad cow disease in Japan. NewScientist.com news service 2005. Published online at http://www.newscientist.com/channel/health/dn6971. Accessed September 2005.

44 Mitchell S. Japan vCJD case poses blood-donor concern. United Press International, June 23, 2005. Published online at http://www.newsdaily.com/index.php?feed=Science&article=UPI-1-20050623-18225000-bc-us-blood.xml. Accessed September 2005.

45 The National Creutzfeldt-Jakob Disease Surveillance Unit UK. Updated November 7, 2005. Published online at http://www.cjd.ed.ac.uk. Accessed November 2005.

別紙様式第2-1
番号1

医薬品
医薬部外品　　研究報告　調査報告書
化粧品

識別番号・報告回数			報告日	第一報入手日 2006年4月21日	新医薬品等の区分 該当なし	厚生労働省処理欄

一般的名称	①②人血清アルブミン ③④乾燥濃縮人アンチトロンビンⅢ ⑤人ハプトグロビン ⑥乾燥濃縮人血液凝固第Ⅷ因子	研究報告の公表状況	J Neurol. Neurosurg. Psychiatry online/ doi:10:1136/jnnp:073395	公表国 イギリス
販売名（企業名）	①献血アルブミン-Wf ②献血アルブミン(5%)-Wf ③ノイアート ④ノイアート静注用1500単位 ⑤ハプトグロビン注-ヨシトミ ⑥コンコエイト-HT			

研究報告の概要

CJDは、孤発型、遺伝型、医原型及び変異型の4つの臨床形態をとる。世界中で最もありふれた形態である孤発型CJDの原因は不明であり、2つの研究が以前に処置された外科的治療によるものと示唆しているものの、症例対照研究は何ら一貫性のある危険要因を確認することができていない。遺伝型CJDは、プリオン蛋白に内在する変異と関係し、これが一般的に直接的な原因と考えられている。しかし変異は多分、現在はまだ認められていないものの、感染源に対しての責任を増している。残り2つのCJDの型は、後天的なものである。変異型CJDは、BSEが原因と考えられ、これは汚染食品によるとされている。医原型CJDは、医療又は外科治療の行為によってCJDの不注意な感染に由来する。医原型CJDの内、2つの最も重要な事例は、死体からのヒトの成長ホルモン治療及び外科手術の際の硬膜移植片の使用によるものである。角膜移植、深部電極及び脳神経外科もまた、まれに関与していた。
硬膜関連CJDの最初の報告は1987年であり、より詳細な報告が翌年に公表された。硬膜関連CJDは現在までに、世界中で164の症例があることが認められている。本報告では、英国におけるサーベイランスで確認された硬膜移植関連CJDの7症例、及び最初のブタ硬膜レシピエントのCJD症例について報告及び記述する。
レシピエントは1988年に右の前頭部髄膜腫の切除術を受けた、そして、豚皮質移植片が硬膜を修復するのに用いられた。レシピエントは、134ヵ月後に頭痛、失調と認識減退を呈した。調査により明らかにされた特徴は、一貫して孤発型CJDであり、典型的な脳波によっても確認され、病理学的診断が下された。検死の結果、前頭および側頭の皮質に海綿状変化を示し、同様の特徴は大脳基底核、視床及び小脳にも認められた。免疫細胞化学検査はPrPの広範囲にわたる蓄積を示し、そして、ウエスタンブロット試験は1型アイソフォームを示した。
我々は、症例VIII（硬膜補修にブタの真皮を使用）がヒト以外の移植片に曝露されたヒトでの最初のCJD感染症例であると考える。発症年齢、潜伏期間、臨床並びに調査の特徴は孤発型CJDの典型例に似ていた。さらに、病理学的特徴もまた、孤発型CJDに特有なものと考えられ、1型PrP^{Pres}と確認された。いずれも、未確認の病原体感染の可能性を完全には排除することができない。しかし、ブタにおけるTSEは、動物モデルでの感染実験による感染でも現在認められていないことから、偶然によるとするのが、最も妥当な説明である。

使用上の注意記載状況・その他参考事項等

代表としてノイアート（献血）の記載を示す。
2. 重要な基本的注意
(1)略
1)略
2)現在までに本剤の投与により変異型クロイツフェルト・ヤコブ病（vCJD）等が伝播したとの報告はない。しかしながら、製造工程において異常プリオンを低減し得るとの報告があるものの、理論的なvCJD等の伝播のリスクを完全には排除できないので、投与の際には患者への説明を十分行い、治療上の必要性を十分検討の上投与すること。

報告企業の意見	今後の対応
英国におけるサーベイランスにおいてブタの真皮を硬膜補修に用いた患者がCJDを発症したとするヒト以外の移植片に暴露されたヒトでの最初のCJD感染症例である。 ブタのTSEは動物実験においても認められていない。また、これまで血漿分画製剤によってスクレイピーを含むプリオン病が伝播したとの報告はない。しかしながら、万一TSE感染動物由来原材料が本剤の原料に混入した場合には、製造工程においてプリオンを低減し得るとの報告があるものの、製剤から伝播する可能性を完全には否定し得ない。そのため、弊社の血漿分画製剤の製造工程におけるTSE感染性低減に関する検証実験を加速し、自社データを早期に取得し、工程評価を行い、必要に応じて工程改善を実施する予定である。	本報告は本剤の安全性に影響を与えないと考えるので、特段の措置はとらない。

ヘパリン

JNNP Online First, published on April 20, 2006 as 10.1136/jnnp.2005.073395

BENE2006-019

SHORT REPORT

Dura mater-associated Creutzfeldt–Jakob disease: experience from surveillance in the UK

C A Heath, R A Barker, T F G Esmonde, P Harvey, R Roberts, P Trend, M W Head, C Smith, J E Bell, J W Ironside, R G Will, R S G Knight

J Neurol Neurosurg Psychiatry 2006;000:1–3. doi: 10.1136/jnnp.2005.073395

Between 1970 and 2003, seven cases of human dura mater-associated Creutzfeldt–Jakob disease (CJD) were identified in the UK. Furthermore, we identified a case of CJD in a porcine dura graft recipient. The mean incubation period of the human dura mater cases was 93 (range 45–177) months. The clinico-pathological features of the cases are described and compared with cases previously reported in the world literature.

Creutzfeldt–Jakob disease (CJD) exists in four clinical forms: sporadic, genetic, iatrogenic and variant. The cause of sporadic CJD, the most common form worldwide, is unknown and case–control studies have failed to identify any consistent risk factor, although two studies have implicated previous surgical interventions.[1 2] Genetic forms of the disease are associated with underlying mutations of the prion protein gene (*PRNP*), which are generally considered to be directly causative.Mutations, however, possibly increase liability to some, as of yet unrecognised, source of infection. The two remaining forms of CJD are acquired. Variant CJD is considered to be caused by bovine spongiform encephalopathy,[3–5] through contaminated food products; iatrogenic CJD results from the inadvertent transmission of CJD during the course of medical or surgical treatment. The two most numerically significant instances of iatrogenic CJD resulted from treatment with cadaveric human growth hormone and the use of dura mater grafts in surgery. Corneal grafts, depth electrodes and neurosurgical instruments have also rarely been implicated.[6 7]

The first report of dura mater-associated CJD was published in 1987,[8] with a more detailed report appearing the following year;[9] to date, 164 cases have been recognised worldwide (P Brown, personal communication). This paper reports and describes the seven cases of human dura mater graft-associated CJD identified during surveillance in the UK and also, for the first time, reports a case of CJD in a porcine dura graft recipient.

METHODS

CJD surveillance in the UK has been undertaken in four phases.

- A retrospective review was carried out in England and Wales from 1970 to 1979.
- A prospective study was carried out in England and Wales from 1980 to 1984.
- A retrospective review was conducted in UK to cover the period from 1985 to 1990.
- A prospective surveillance was instituted in the UK in 1990 and continues.

The methodology of the National CJD Surveillance Unit has been described in previous publications.[10 11]

RESULTS

Human dura mater

During the period between 1970 and 2003, seven cases of human dura mater-associated CJD were identified in the UK. Table 1 shows the basic demographic features. The latent period between surgery and the onset of CJD ranged from 45 to 177 (mean: 93) months. The mean age at surgery was 33 years, with a mean age at onset of 41 years.

Lyodura (B Braun Melsungen, Germany), a particular brand of human dura mater, was implicated in six of the seven cases (the manufacturer of the dura graft implicated in case I is unknown).

The six cases associated with Lyodura were exposed to the presumed source of "infection" between 1983 and 1987, with the first recognised case in the UK exposed to human dura mater in 1969.

A detailed account of both clinical and investigative features is available online. In four cases, the clinical phenotype at onset appears to correlate with the site of graft placement or underlying parenchymal damage (cases II, III, V and VI). For example—in case II, the initial illness presentation included a right visual field defect; a left hemisphere tumour was also diagnosed. The subsequent CJD began with a right visual field disturbance and progressed with signs indicating the involvement of the left hemisphere. Some of the cases were investigated before the widespread availability of MRI, and therefore MRI was only available in only four of the seven cases. None of the cases showed the characteristic radiological features of human prion disease[14] with post-surgical change being the most commonly recognised abnormality. Despite all seven cases having at least one electroencephalogram during the course of investigation, only three of the seven cases showed the "typical" features.

Autopsy was carried out in five of our seven cases. In general, the neuropathology was characterised by widespread spongiform change accompanied by variable neuronal loss and gliosis. Western blot analysis for PrP^{res} was carried out in three cases (cases II, VI and VII). The mobility and glycoform ratio of the PrP^{res} is indistinguishable from those of the type 1 PrP^{res}, identified in cases of sporadic CJD, and is distinct from type 2B, PrP^{res} identified in variant CJD.

Porcine dura mater

We believe the identification of CJD in a porcine graft recipient to be the first such report worldwide (table 1,case VIII). The recipient underwent excision of a right fronto-

Abbreviations: CJD, Creytzfeldt–Jakob disease

Case V received two dura grafts, is assumed that the first graft was responsible for transmission.

Table 1 UK case details—human dura mater

Case	Surgical procedure	Dura	Year of surgery	Year of death	Incubation period (Months)	Duration of illness (Months)
I, Esmonde *et al*[12]	Suboccipital craniotomy and C1/2 laminectomy for cerebellar ectopia and syringomyelia	?	1969	1979	104*	6
II, Esmonde *et al*[12]	Excision of a left temporal cortex meningioma	L	1983	1991	93*	5*
III	Repair surgical leak after acoustic neuroma excision	L	1985	1989	51	2
IV, Willison *et al*[13]	Posterior fossa decompression and cervical laminectomy for cerebellar ectopia or syringomyelia	L	1985	1989	45*	4
V	Excision of a left parietal cortex meningioma	1) L	1985	1993	1) 86	11
		2) L	1986		2) 79	
VI	Excision of a cerebellar astrocytoma	L	1986	1997	103	33
VII	Excision of a eosinophilic granuloma right frontal region skull	L	1987	2003	177	5
Porcine Dura Graft:						
VIII	Excision of a right frontoparietal meningioma	P	1988	2000	134	3

*Revised from previously published figures.
L, Lyodura; P, Porcine dura.

parietal meningioma in 1988 and a zenoderm graft was used to repair the dura. The recipient presented with headaches, ataxia and cognitive decline after 134 months. Investigative features were consistent sporadic CJD, with a typical electroencephalogram was identified, and pathological confirmation was obtained. Autopsy showed spongiform change in the frontal and temporal cortex, with similar features identified in the basal ganglia, thalamus and cerebellum. Immunocytochemistry for PrP showed widespread accumulation and western blot analysis showed the type 1 isoform.

DISCUSSION

Human dura mater is a rare, but important source of transmission of human prion disease, with only seven cases recognised in a 33-year period. Surveillance systems worldwide have identified 164 cases of CJD in people previously exposed to human dura mater. Prevalence is particularly high in Japan and probably reflects neurosurgical practice, with an estimated 20 000 grafts used each year.[15] The overall risk of CJD associated with human dura grafts in the UK is unknown because an accurate estimation of human dura graft use and thus a denominator for calculation of risk is not available. The estimated risk after exposure in Japan has been estimated to be approximately 1 per 2000 patients treated between 1979 and 2000 and approximately 1 per 1000 between 1983 and 1987.[16] Neurosurgical practice in Japan, with widespread use of dura mater, may be different from other countries throughout the industrialised world and therefore it would seem unreasonable to extrapolate any estimated risk from these data. If neurosurgical practices in the UK were more akin to those in Australia, then a subsequent study by Brooke and co-workers would help provide additional information pertaining to estimated risk. By using information from the Australian CJD Surveillance system, Brooke and co-workers estimated the risk associated with exposure to human dura mater to be approximately 1 per 500 patients treated between 1978 and 2003.[17] Clearly, the risk of developing CJD in this patient population is considerably higher than we would expect by chance.

The human dura mater implicated in the transmission of CJD was processed, almost exclusively, by B Braun Melsungen in Germany and traded under the name Lyodura. Over 100 Japanese cases, and all but one of the UK cases (the source of the first case identified in the UK is unknown), have been associated with this particular product and only rarely has dura processed by other manufacturers been associated with transmission.[18 19] Although the first case in the UK was exposed to potentially infectious dura in 1969, a disproportionately large number of cases were exposed between 1983 and 1987 (80% of those identified worldwide and six of the seven cases in the UK). Interestingly, the apparent reduction in the number of cases post-1987 coincided with the introduction of stringent donor selection criteria and also the introduction of sodium hydroxide immersion techniques in the manufacturing process.

We found no temporal or geographical association between any of the dura-associated cases, or any other case of CJD identified in the UK, despite potential contamination of neurosurgical instruments.

It has been proposed that clinical features at onset are dependent on the site of graft placement or underlying parenchymal damage[20–22] and our findings may support such a proposition. The explanation for this observation is unclear. We, could, however, speculate that the pathological process starts within a region adjacent to the graft and that this is reflected in the early clinical features. This proposition may also be supported by findings obtained at autopsy, with severe pathological changes identified adjacent to graft placement in three cases. Overall, the pathology is consistent for that previously described in dura mater-associated CJD.[9 18] We did not identify either "kuru-type" or florid PrP plaques. The florid PrP plaques were previously noted in limited distribution in a small number of dural graft-associated iatrogenic CJD cases in Japan.[21 23 24]

We believe case VIII represents the first reported case of CJD in a person previously exposed to a graft from a non-human source. The age at onset, duration of illness, clinical and investigative features were similar to a typical case of sporadic CJD. Furthermore, the pathological features were also considered characteristic of sporadic CJD, with type 1 PrP^{res} identified. Neither finding can definitively exclude the possibility of transmission of a yet unidentified pathogen. As natural transmissible spongiform encephalopathies are, however, as yet unrecognised in pigs, despite experimental transmission in animal models,[25] a chance association seems the most plausible explanation.

ACKNOWLEDGEMENTS

We thank Jan MacKenzie for all her help in producing this manuscript. We also thank our colleagues within the European

surveillance programme for updated information, particularly Pascual Sanchez Jaun, and neurological colleagues throughout UK, particularly Dr Graham Lennox, for assistance with this manuscript. Finally, we thank our colleagues at the NCJDSU, particularly Alison Green, for carrying out CSF analysis, David Summers for advice on neuroimaging, and also Hester Ward and Katy Murray for comments on the manuscript.

Authors' affiliations
C A Heath, M W Head, C Smith, J E Bell, J W Ironside, R G Will, R S G Knight, National CJD Surveillance Unit, University of Edinburgh, Edinburgh, UK
R A Barker, Cambridge Centre for Brain Repair, University of Cambridge, Cambridge, UK
T F G Esmonde, Royal Victoria Hospital, Belfast, UK
P Harvey, Harley Street, London, UK
R Roberts, Ninewells Hospital, Dundee, UK
P Trend, The Royal Surrey County Hospital, Surrey, UK

Competing interests: None.

Correspondence to: Dr R S G Knight, National CJD Surveillance Unit, Bryan Matthews Building, Western General Hospital, Edinburgh EH4 2XU, UK; r.knight@ed.ac.uk

Received 7 June 2005
Revised version received 23 August 2005
Accepted for publication 24 August 2005

REFERENCES

1 **Collins S,** Law MG, Fletcher A, *et al.* Surgical treatment and risk of sporadic Creutzfeldt-Jakob disease: a case-control study. *Lancet* 1999;353:693–7.
2 **Ward HJT,** Everington D, Croes EA, *et al.* Sporadic Creutzfeldt-Jakob disease and surgery: a case-control study using community controls. *Neurology* 2002;59:543–8.
3 **Will RG,** Ironside JW, Zeidler M, *et al.* A new variant of Creutzfeldt-Jakob disease in the UK. *Lancet* 1996;347:921–5.
4 **Collinge J,** Sidle KCL, Meads J, *et al.* Molecular analysis of prion strain variation and the aetiology of 'new variant' CJD. *Nature* 1996;383:685–90.
5 **Bruce ME,** Will RG, Ironside JW, *et al.* Transmissions to mice indicate that 'new variant' CJD is caused by the BSE agent. *Nature* 1997;389:498–501.
6 **Duffy P,** Wolf J, Collins G, *et al.* Possible person-to-person transmission of Creutzfeldt-Jakob disease. *New Engl J Med* 1974;290:692–3.
7 **Foncin JF,** Gaches J, Cathala F, *et al.* Transmission iatrogene interhumaine possible de maladie de Creutzfeldt-Jakob avec atteinte des grains de cervelet. *Rev Neurol* 1980;136:280.
8 **Prichard JW,** Thadani V, Kalb R, *et al.* Rapidly progressive dementia in a patient who received a cadaveric dura mater graft. *Morb Mortal Wkly Report* 1987;34:49–55.
9 **Thadani V,** Penar PL, Partington J, *et al.* Creutzfeldt-Jakob disease probably acquired from a cadaveric dura mater graft. *J Neurosurg* 1988;69:766–9.
10 **Will RG.** A new variant of Creutzfeldt-Jakob disease in the UK. *Proceedings of the Society for Veterinary Epidemiology and Preventative Medicine* 1997.
11 **Will RG,** Zeidler M, Stewart GE, *et al.* Diagnosis of new variant Creutzfeldt-Jakob disease. *Ann Neurol* 2000;47:575–82.
12 **Esmonde T,** Leuck CJS, Duchen LW, *et al.* CJD and lyophilised dura mater grafts: a report of two cases. JNNP 1991;56:999–1000.
13 **Willison HJ,** Gale AN, McLaughlin JE. Creutzfeldt-Jakob disease following cadaveric dura mater graft. *JNNP* 1991;54:940.
14 **Collie DA,** Sellar RJ, Zeidler M, *et al.* MRI of Creutzfeldt-Jakob disease: imaging features and recommended MRI protocol. *Clin Radiol* 2001;56:726–39.
15 **Tateishi J.** CJD transmitted through cadaveric dura grafts. *J Clin Exp Med* 1997:992–3.
16 **Nakamura Y,** Watanabe M, Nagoshi K. CJD associated with cadaveric dura mater grafts-Japan 1979–2003. *Morb Mortal Wkly Report* 2005;52:1179–81.
17 **Brooke FJ,** Boyd A, Klug GM, *et al.* Lyodura use and the risk of iatrogenic Creutzfeldt-Jakob disease in Australia. *Med J Aus* 2004;180:1–5.
18 **Pocchiari M,** Masullo C, Salvatore M, *et al.* Creutfeldt-Jakob disease after non-commercial dura mater graft. *Lancet* 1992;340:614–5.
19 **Hannah EL,** Belay ED, Gambetti P, *et al.* Creutzfeldt-Jakob disease after receipt of a previously unimplicated brand of dura mater graft. *Neurology* 2001;56:1080–3.
20 **Sato T.** CJD with dura mater transplantation. *Clin Neurol* 2003;43:870–2.
21 **Fushimi M,** Sato K, Shimizu T, *et al.* PLEDS in Creutzfeldt Jakob disease following a cadaveric dura graft. *Clin Neurophysiol* 2002;113:1030–5.
22 **Nishida y, Yamane M, Hara k, et al.** Creutzfeldt-Jakob disease after Jannetta's operation with cadaveric dura mater. *J Neurol* 2002;249:480–3.
23 **Takashima S,** Tateishi J, Taguchi Y, *et al.* Creutzfeldt-Jakob disease with florid plaques after cadaveric dural graft in a Japanese woman. *Lancet* 1997;350:865–6.
24 **Kimura K,** Nonaka A, Tashiro H, *et al.* Atypical form of dural graft associated Creutzfeldt-Jakob disease: report of a postmortem case with review of the literature. *JNNP* 2001;70:696–9.
25 **Castilla J,** Gutierrez-Adan A, Brun A, *et al.* Subclinical BSE infection in transgenic mice expressing porcine prion protein. *J Neurosci* 2004;24:5063–9.

別紙様式第 2-1
番号 14

医薬品
医薬部外品　　研究報告　調査報告書
化粧品

識別番号・報告回数			報告日	第一報入手日 2006 年 6 月 26 日	新医薬品等の区分 該当なし	厚生労働省処理欄
一般的名称	①②ポリエチレングリコール処理人免疫グロブリン ③人免疫グロブリン		研究報告の公表状況	The Lancet 2006; 367(9528):2068-2074	公表国 パプアニューギニア	
販売名 (企業名)	①献血ヴェノグロブリン-IH ヨシトミ（ベネシス） ②ヴェノグロブリン-IH（ベネシス） ③グロブリン-Wf（ベネシス）					

研究報告の概要

＜背景＞
クールー病は、ヒトの流行性プリオン病について重要な経験を提供している。その発生は、1950 年代にパプアニューギニアでの感染ルート（部族内食人）の突然の中断の後、着実に減っていった。vCJD が発生し、英国において食事により広範囲に BSE プリオンへの曝露された後の感染率は不明であるために、クールー病が再び関心を呼ぶことになった。我々は、パプアニューギニアのクールー病患者における、潜伏期間、病因及び感染しやすさについての遺伝的要因について調査した。
＜方法＞
我々は、全ての疑わしい患者を調査するために、調査チームの規模を広げるとともに 1996 年にクールー病の監視を強化した。住民の居住歴と葬儀の宴での曝露歴の情報が、一連の神経学的検査とともに、可能な限り集められた。
＜結果＞
我々は、1996 年 7 月から 2004 年 6 月までに 11 人のクールー病患者を確認したが、全員が South Fore に住んでいた。患者は全員、1950 年代後半に食人習慣が中止される前に生れていた。最短の推定潜伏期間は、34 年から 41 年の範囲であった。しかし、男性における潜伏期間は 39 年から 56 年の範囲と考えられたが、実際はこれより最長で 7 年長かった可能性がある。プリオン蛋白の分析によって、殆どのクールー病の患者は、潜伏期間の延長とプリオン病への抵抗性に関連づけられている多形コドン 129 がヘテロ接合体の遺伝子型であった。
＜解釈＞
ヒトのプリオンに感染した場合の潜伏期間は、50 年を超える可能性がある。BSE プリオンにヒトが感染した場合、種を超えた場合の感染の特徴である「種の壁の効果」が、同じ種の中でのプリオンの感染と比較して、潜伏期間の平均値と範囲をさらに大きくするであろう。これらのデータは、vCJD の疫学モデルの作成の試みに影響を与えるはずである。

使用上の注意記載状況・その他参考事項等

代表として献血ヴェノグロブリン-IH ヨシトミの記載を示す。
2. 重要な基本的注意
(1)略
1)略
2)現在までに本剤の投与により変異型クロイツフェルト・ヤコブ病（vCJD）等が伝播したとの報告はない。しかしながら、製造工程において異常プリオンを低減し得るとの報告があるものの、理論的な vCJD 等の伝播のリスクを完全には排除できないので、投与の際には患者への説明を十分行い、治療上の必要性を十分検討の上投与すること。

報告企業の意見	今後の対応
パプアニューギニアのクールー病患者の研究から、ヒトのプリオンに感染した場合の潜伏期間は、50年を超える可能性があることを示唆した報告である。 これまで血漿分画製剤によってvCJDを含むプリオン病が伝播したとの報告はない。しかしながら、万一vCJD感染者の血漿が本剤の原料に混入した場合には、製造工程においてプリオンを低減し得るとの報告があるものの、製剤から伝播する可能性を完全には否定し得ない。そのため、弊社の血漿分画製剤の製造工程におけるTSE感染性低減に関する検証実験を加速し、自社データを早期に取得し、工程評価を行い、必要に応じて工程改善を実施する予定である。	vCJD の疫学情報については、今後も注視することとする。

Kuru in the 21st century—an acquired human prion disease with very long incubation periods

John Collinge, Jerome Whitfield, Edward McKintosh, John Beck, Simon Mead, Dafydd J Thomas, Michael P Alpers

Lancet 2006; 367: 2068–74

MRC Prion Unit and Department of Neurodegenerative Disease, Institute of Neurology, University College London, London WC1N 3BG, UK (Prof J Collinge FRS, J Whitfield MSc, E McKintosh MRCS, J Beck BSc, S Mead MRCP, D J Thomas FRCP, M P Alpers FTWAS); Papua New Guinea Institute of Medical Research, Goroka, EHP, Papua New Guinea (J Whitfield); and Centre for International Health, Curtin University, Perth, Australia (M P Alpers)

Correspondence to: Prof John Collinge
j.collinge@prion.ucl.ac.uk

Summary

Background Kuru provides the principal experience of epidemic human prion disease. Its incidence has steadily fallen after the abrupt cessation of its route of transmission (endocannibalism) in Papua New Guinea in the 1950s. The onset of variant Creutzfeldt-Jakob disease (vCJD), and the unknown prevalence of infection after the extensive dietary exposure to bovine spongiform encephalopathy (BSE) prions in the UK, has led to renewed interest in kuru. We investigated possible incubation periods, pathogenesis, and genetic susceptibility factors in kuru patients in Papua New Guinea.

Methods We strengthened active kuru surveillance in 1996 with an expanded field team to investigate all suspected patients. Detailed histories of residence and exposure to mortuary feasts were obtained together with serial neurological examination, if possible.

Findings We identified 11 patients with kuru from July, 1996, to June, 2004, all living in the South Fore. All patients were born before the cessation of cannibalism in the late 1950s. The minimum estimated incubation periods ranged from 34 to 41 years. However, likely incubation periods in men ranged from 39 to 56 years and could have been up to 7 years longer. *PRNP* analysis showed that most patients with kuru were heterozygous at polymorphic codon 129, a genotype associated with extended incubation periods and resistance to prion disease.

Interpretation Incubation periods of infection with human prions can exceed 50 years. In human infection with BSE prions, species-barrier effects, which are characteristic of cross-species transmission, would be expected to further increase the mean and range of incubation periods, compared with recycling of prions within species. These data should inform attempts to model variant CJD epidemiology.

Kuru is one of a group of closely related neurodegenerative conditions that affect both human beings and animals, known as the transmissible spongiform encephalopathies or prion diseases.[1] Prion diseases are associated with the accumulation in the brain of an abnormal, partly protease-resistant, isoform of a host-encoded glycoprotein known as prion protein (PrP). According to the protein-only hypothesis, an abnormal PrP isoform is the main, and possibly the only, constituent of the transmissible agent or prion.

The large-scale epidemic of bovine spongiform encephalopathy (BSE) in the UK led to fears of a serious threat to public health. Since 1996, cases of a novel human prion disease, variant Creutzfeldt-Jakob disease (vCJD), have been identified in the UK, and strain typing has confirmed that both vCJD and BSE are caused by the same prion strain.[1] Dietary exposure of the UK population to BSE prions has been widespread; the total cattle epidemic is thought to have affected 2 million cows.[2] Cattle BSE has also been reported in most EU states, Israel, Switzerland, Canada, the USA, and Japan. So far, about 160 vCJD patients have been identified in the UK, with cases also reported in France, Italy, Ireland, the Netherlands, Canada, Japan, and the USA. Predictions of the eventual size of a vCJD epidemic have varied widely, although some recent estimates, based on current cases of vCJD, suggest that the total epidemic may be relatively small.[3] However, key uncertainties, notably with respect to major genetic effects on the incubation period,[4] suggest the need for caution. Importantly, such models cannot estimate the number of infected individuals, which remains unknown, and concerns of secondary transmission have heightened.[5] These uncertainties, especially with the possibility of very long incubation periods of BSE in people, have renewed interest in kuru, which remains the only example of a major human epidemic.

Kuru reached epidemic proportions in a defined population of the Eastern Highlands of Papua New Guinea. Local oral history, taken when the disease was first studied by western medicine in the 1950s, dated the onset of the first cases to the 1920s. Kuru mainly affected the people of the Fore linguistic group and also their neighbours with whom they intermarried. The disease predominantly affected women and children (of both sexes), with only 2% of cases in adult men;[6] kuru also became the most common cause of death in women in affected villages. Kuru is a cerebellar syndrome with a characteristic and relentless progression through defined clinical stages, and is invariably fatal. Cognition is fairly preserved, and the disease is highly distinctive and is usually recognised easily by both the patients and their local community.[6]

These communities practised the consumption ritual of dead relatives as a mark of respect and mourning. Boys older than 6–8 years participated little in mortuary

feasting, which could explain the differential age and sex incidence. From the age of the youngest affected patient, the shortest incubation period is estimated to be 5 years, although this time could be shorter, since time of infection is usually unknown.

Genetic susceptibility is important in both the sporadic and acquired forms of human prion disease; human PrP has a common polymorphism, with either methionine or valine present at residue 129. About 38% of Europeans are homozygous for the more frequent methionine allele, 51% are heterozygous, and 11% homozygous for valine. Most sporadic CJD occurs in individuals homozygous for this polymorphism.[7] This susceptibility factor is also relevant in the acquired forms of CJD, most strikingly in vCJD; all clinical cases studied so far have been homozygous for codon 129 methionine of the PrP gene *PRNP*.[5] The *PRNP* codon 129 genotype has shown a pronounced effect on kuru incubation periods and susceptibility, and most elderly survivors of the kuru epidemic are heterozygotes.[8,9] The clear survival advantage for codon 129 heterozygotes provides a powerful basis for selection pressure in the Fore. However, an analysis of worldwide haplotype diversity and allele frequency of coding and non-coding polymorphisms of *PRNP* suggests that balancing selection at this locus (in which there is more variation than expected because of heterozygote advantage) is much older and more geographically widespread. Evidence for balancing selection has been shown in only a few human genes. With biochemical and physical evidence of cannibalism on five continents, one explanation is that cannibalism resulted in several prion disease epidemics in human prehistory, thus imposing balancing selection on *PRNP*.[9]

Kuru was extensively studied at its peak in the late 1950s and early 1960s and monitoring was continued through the Papua New Guinea Institute of Medical Research. We strengthened active kuru surveillance in 1996 and aimed to study all patients with kuru until the end of the epidemic. Here, we aimed to determine the maximum period possible for incubation in human prion infection and to investigate genetic factors in recent patients with kuru. The abrupt interruption of transmission of kuru, after the effective prohibition of cannibalism by Australian authorities in the mid-1950s, allows a unique opportunity to investigate the incubation period of infection as a key variable in human prion disease. Our findings of detailed clinical features and mortuary feast practices will be reported elsewhere.

Methods

Research ethics

Our study was approved by the Papua New Guinea Medical Research Advisory Committee and by the local research ethics committees of St Mary's Hospital and National Hospital for Neurology and Neurosurgery, in London, UK. The full participation in the project from the communities, which was critical with respect to the ethics and operation of the study, was established and maintained through discussions with village leaders, communities, families, and individuals, and the field studies followed the principles and practice of the Papua New Guinea Institute of Medical Research.

Kuru surveillance and clinical studies

A field base and laboratory was established in Waisa in the South Fore. A team of local kuru reporters communicated details of any suspected case to the field base. 50 suspect cases investigated during this period proved not to have kuru. The field team consisted of staff from the UK Medical Research Council (MRC), the Papua New Guinea Institute of Medical Research, and local communities, who undertook regular field patrols throughout the kuru-

	Sex	Year of birth	Onset	Age at onset (years)	Age at death (years)	*PRNP* 129 genotype	Minimum incubation period (years)*	Likely incubation period (years)†
PKW	F	1946	August, 1995	49	50	Heterozygous	35	..
YAK	M	1948	November, 1994	46	48	Heterozygous	34	39
MWK	M	1933	April, 1996	63	64	Methionine homozygous	36	56
AKA	M	1949	November, 1996	47	49	Heterozygous	36	40
AYA	M	1936	November, 1998	62	63	n/a	38	55
TAM	F	1945	March, 1999	54	55	Valine homozygous	39	..
AYY	M	1940	June, 1998	58	60	Heterozygous	38	51
WKW	M	1943	January, 1999	56	57	Heterozygous	39	49
MAA	F	1944	April, 1999	55	57	Heterozygous	39	..
INO	F	1942	January, 2000	58	59	Heterozygous	40	..
KAW	M	1943	October, 2001	58	60	Heterozygous	41	51

Patients' initials are based on name and coded. *Calculated conservatively as the number of years between 1960 and onset of kuru (assuming latest possible exposure at mortuary feast in 1959). This period would be an underestimate (in some cases of many years) of the actual incubation period (measured from the date that infection was actually acquired). The maximum incubation period possible (in the event of neonatal infection) would be the same as age at onset. †Since male individuals were unlikely to be infected after age 6–8 years, the likely minimum incubation period can be calculated as the number of years from age 7 years to disease onset, which is also a conservative estimate, since actual infection could have taken place (up to 7 years) earlier. F=female. M=male. n/a=not available

Table 1: Estimation of kuru incubation periods in 11 patients identified in current study

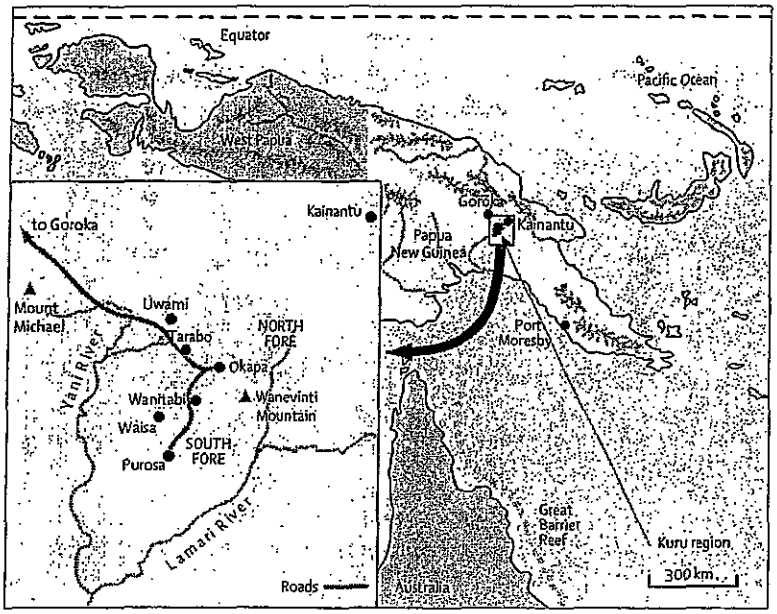


Figure 1: Area and Fore groups historically affected by kuru in the Eastern Highlands Province of Papua New Guinea

affected area, which included the North and South Fore, Keiagana, Kanite, and Gimi linguistic groups. The fieldworkers had to work under very difficult conditions during the surveillance, and heavy-vehicle rescue equipment and a six-man team were needed at times to proceed with the project. Security issues intermittently affected our ability to travel to and from the field site. Regular field neurological examinations were done when possible and recorded by video and photography. Case histories and patterns of exposure to mortuary feasts were also documented.

Molecular genetic studies

We extracted genomic DNA from venous blood to determine the complete coding sequence of *PRNP* (PrP gene).[10] Papua New Guinean control genotype data were obtained by restriction endonuclease digestion of PCR amplicons (for comparison with *APOE* [apolipoprotein E] and *PRND* [Doppel, a prion-protein-like protein] genes), and allelic discrimination with the ABI SDS7000 sequence detection system (for comparison with codon 129 polymorphisms and haplotypes of *PRNP*). We identified HLA-DQB1 alleles by automated fluorescent sequencing of PCR amplicons using the Amersham MEGAbace DNA analysis system (Amersham, UK).

Role of the funding source

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

Results

The total number of cases of kuru from 1957 to 2004 exceeded 2700, with more than 200 dying every year in the late 1950s. This number fell to about six a year in the early 1990s and between one and two a year during the study; since July, 1996, we identified 11 kuru patients up to the end of June, 2004.

Table 1 shows the ages at onset of all 11 patients in the study. Age might not be accurately known or reported by individuals in these communities, but can be reliably and accurately estimated by reference to family relationships and clearly defined recent historical events.[11] Figures 1 and 2 show the area and populations historically affected by kuru. All patients identified in the current study were from the South Fore, now that kuru has disappeared from the North Fore and adjacent linguistic groups (figure 2).

On the basis of a conjunction of experimental, epidemiological, and human behavioural evidence,[12] we can assume that all transmission through the traditional mortuary practice had ceased by 1960. The practice had been important to the Fore people as a way of respecting their dead relatives, but it was rigorously forbidden by Australian Government officers in one of their first acts of administrative control after making contact with the people, when the Okapa patrol post was established in 1954. Public consumption of dead relatives ceased almost immediately and compliance was ensured by the police force responsible for the subdistrict. By 1956, endocannibalism was effectively gone. Surreptitious eating of dead relatives had been reported in remote communities for some years afterwards, but by the end of the 1950s the practice had ended. Epidemiological surveillance for kuru began in 1957 and has been continued ever since. Because of the wide geographical extent of the families participating in a feast, secret feasting with entire families taking part would not have gone undetected. The communities of the North Fore, who had been the first of the Fore people to lose their traditional practices in the wake of Australian administrative control, ended their mortuary feasting at the beginning of the decade or earlier; kuru is no longer present in this area. The latest year of birth recorded for any patient with kuru is 1959; only nine patients are recorded as being born since 1956. These individuals were mostly young patients and their ages at onset could have been underestimated by a few years. Therefore, for practical purposes, we can assume that all transmission through the traditional mortuary practices had ceased by 1960.

Therefore, we could define the minimum incubation period as the time between 1960 and the date of onset of kuru (table 1). For patients born in earlier decades, the actual incubation could be much longer; however, since the infecting event can never be known, all individuals alive during the period when consumption of dead relatives' bodies was universally practised must be regarded as being at risk.

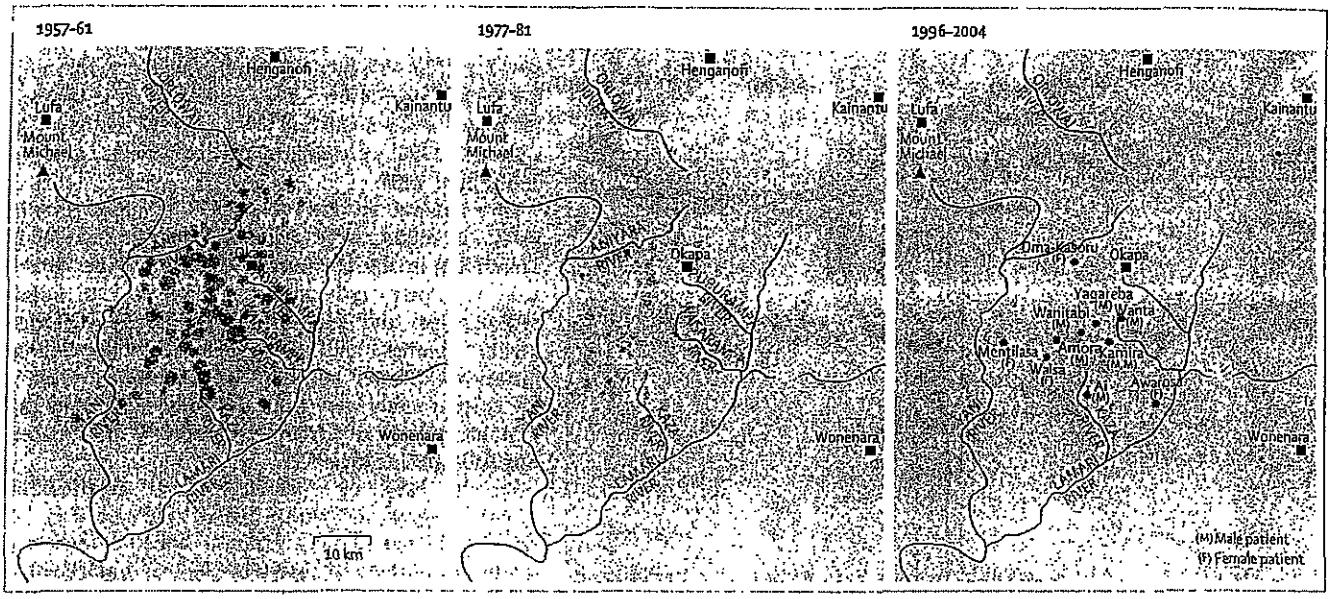


Figure 2: Individual patients with kuru recorded during the 5 years immediately after cessation of endocannibalism (1957–61), after 20 years (1977–81), and in the current study (1996–2004)
Patients plotted by village of residence. Black squares indicate towns.

After the age of 6–8 years, boys were taken from their mothers and brought up in the men's house. From this point on, they were exposed only to the same risk as adult men, who participated little in feasts and did not eat the brain, by far the most infectious organ in kuru. This practice can explain why adult men in 1957–58 contributed only 2% to the total number of kuru cases; and from what we now know about the incubation period, most would have been from transmissions in their childhood. Therefore, we can estimate that the likely incubation period of the male patients identified in this study began at about age 7 years, lasting up to the age of onset (table 1). These projections are less robust than the minimum estimates, but nevertheless are potentially real and should not be ignored in any consideration of the incubation period of human prion diseases.

	PRNP haplotype	*APOE*	*PRND* 174 genotype	*HLA-DQB1*
PKW	AF	E2,E3	MT	*050301/*0602
YAK	AB	E2,E3	MT	*0401/NR
MWK	FF	E3,E3	TT	*050301/*050201
AKA	AF	E4,E4	MT	*050301/*030101
AYA	n/a	n/a	n/a	n/a
TAM	AA	E3,E3	TT	*0602/*0602
AYY	AF	E3,E3	TT	*050301/*0602
WKW	AF	E3,E3	TT	*0602/*0602
MAA	AF	E2,E3	TT	*050301/*0602
INO	AF	E2,E4	TT	*050301/*0602
KAW	AF1 or A1F	E3,E4	TT	*050301/*0602

Patient's initials are based on name and coded. NR=allele not recognised. n/a=not available. MT=methionine-threonine heterozygous. *APOE*, *PRND* 174, and *HLA-DQB1* are genetic loci implicated in human prion disease susceptibility.

Table 2: Genetic analysis in kuru patients identified in current study

DNA was available for genetic analysis from ten of 11 patients with kuru (table 2). Eight patients were heterozygous at the polymorphic residue 129 of *PRNP*. In 140 unrelated healthy individuals from the South Fore, allele frequencies at the *PRNP* residue 129 were 48% methionine and 52% valine. Although most of the kuru cases with long incubation periods were heterozygous (as expected), this distribution did not differ from the current frequencies of the control population (χ^2 test, p=0·22). The *PRNP* B haplotype has been associated with susceptibility to sporadic CJD in the UK,[20] and *PRNP* haplotyping was also done in these patients and in the healthy Fore population (table 2). Although the analysis had a small sample size, *PRNP* haplotypes of kuru patients with long incubation periods did not differ significantly from the healthy South Fore population (χ^2 test, p=0·85). For other genetic loci implicated in human prion disease susceptibility (*PRND*,[14–15] *APOE*,[16,17] and *HLA*;[18,19] table 2), these alleles for kuru patients with long incubation periods also were not significantly different from the healthy population in the South Fore.

Discussion

The early clinical, epidemiological, and anthropological study of kuru; the recognition of its neuropathological, and then causal parallels to ovine scrapie;[20] and then crucially, the experimental transmission of the disease

to primates,[21] originated the concept of the human transmissible spongiform encephalopathies, which was followed in turn by the eventual unifying concept of the mammalian prion diseases. However, in addition to the central historical importance of kuru, study of the end-stage of this epidemic offers a unique opportunity to study the variables of a near-complete epidemic of human prion disease. In particular, recognition of the incubation periods possible after natural prion infection in people is important in providing an insight (from actual case histories rather than from mathematical models) into the probable span of the vCJD epidemic in the UK. Although estimation of kuru incubation periods early in the epidemic was difficult, and the timing of the actual infecting event for an individual can rarely be determined, the abrupt and permanent interruption of the source of infection, endocannibalism, in the late 1950s, has progressively allowed recognition of an enormous span of possible incubation periods, at its shortest extreme bracketed by the rare onset of disease in children as young as 5 years and extending up to (and perhaps beyond) the incubations covering more than half a century, as we describe here.

In our field studies, we have interviewed many individuals who participated in traditional mortuary feasting or who described the participation of family members from the preceding generation. These detailed descriptions will be published elsewhere but have reaffirmed the oral histories of endocannibalism in the Fore recorded previously[11,22–24] and that this practice ceased abruptly at the time of Australian administrative control over the kuru areas. Although isolated events might have occurred for a few years after this prohibition, we are confident that new exposures of individuals to kuru at mortuary feasts would not have occurred after 1960. Not only have no cases of kuru been recorded in people born after 1959 (and only nine were recorded in those born after 1956); but also all the 11 last recorded cases of kuru that we report here were born before 1950. If any source of infection remained, whether from surreptitious cannibalism, possible ground contamination with human prions at sites where food was prepared, or other lateral routes, we would expect individuals born after this period to have kuru—especially since children are thought to have had shorter incubation periods than adults. However, no such cases have been observed.

Additionally, although a fraction of hamster-adapted scrapie prions have been shown to survive in soil for at least 3 years,[25] the mortuary feast practices (during which the entire body would be consumed) were undertaken so that any substantial contamination of soil would not have occurred, and traditional bamboo knives and leaf plates were burned after the feast. Furthermore, no clusters of kuru cases, as seen earlier in the epidemic,[26] have been recorded for many years. We have also reviewed the assertion that maternal transmission of kuru did not occur, and saw no evidence for maternal transmission from kuru archives, interviews of colleagues who have practised medicine in the Fore, or local oral history. Again, any possible vertical route of kuru transmission would have resulted in the presence of kuru in children born after 1960, especially since kuru was common in women of childbearing age; no such cases have occurred.

With respect to extrapolation of incubation periods of BSE prion infection in people, we should recognise that the kuru epidemic arose from intraspecies recycling of infectious prions. However, transmission of prions between different mammalian species is associated with a species barrier, which is better described as a transmission barrier, because of the importance of within-species prion strain type, in addition to species-specific differences in its determination.[27] The biological effects of such a barrier are: extended mean incubation period; increased spread of incubation periods in individual animals; and reduced attack rate (in which only a fraction of inoculated animals will succumb), by comparison with the 100% mortality generally associated with within-species inoculation with high-titre infectivity. Incubation periods approaching the natural lifespan of the inoculated species are often seen in such primary cross-species transmissions of prions. Second and subsequent passage of prions within the new species is always associated with adaptation involving a considerable shortening of the mean and spread of incubation periods and high or total lethality to high-titre inocula. Thus, estimation of the range of possible incubation periods in human BSE infection needs superimposition of the effect of a transmission barrier onto these findings of natural human incubation periods.

The mean incubation period for kuru has been estimated to be around 12 years,[17] with a similar estimate in iatrogenic CJD associated with the use of human-cadaver-derived pituitary growth hormone.[28] As shown here, maximum incubation periods in kuru can exceed 50 years. The transmission barrier of BSE between cattle and human beings is unknown and cannot be directly measured. However, the cattle-to-mouse barrier for BSE has been well characterised experimentally by comparative endpoint titration. BSE prions transmit readily to laboratory mice, including after oral dosing.[29] The murine LD_{50} (lethal dose causing 50% mortality) in C57Bl/6 mice is about 500-fold higher than that in cattle;[10] this barrier also results in a three-fold to four-fold increase in mean incubation period.[27] Mean incubation periods of human BSE infection of 30 years or more should therefore be regarded as possible, if not probable,[27] with the longest incubation periods approaching (and perhaps exceeding) the typical human lifespan. The shortest incubation periods in kuru were estimated from the age of the youngest patients—suggesting that the shortest incubation period was

4–5 years. Similarly in vCJD, although the total clinical caseload so far has been small, the youngest onsets of vCJD have been at age 12 years or above, providing an early estimate of a minimum incubation period.

Furthermore, prion disease in mice follows a well-defined course with a highly distinctive and repeatable incubation time for a specific prion strain in a defined inbred mouse line. In addition to the PrP gene, a few additional genetic loci with a major effect on incubation period have been mapped.[4,31,32] Human homologues of such loci could be important in human susceptibility to prion disease, both after accidental human prion exposure and after exposure to the BSE agent. By definition, patients identified so far with vCJD are those with the shortest incubation periods for BSE. These patients could have received an especially high dose of BSE prions. However, no unusual history of dietary, occupational, or other exposure to BSE has been reported from case-control studies. Because of the powerful genetic effects on incubation period in laboratory animals, vCJD patients identified could represent a distinct genetic subpopulation with unusually short incubation periods to BSE prions, with vCJD so far occurring predominantly in those individuals with short incubation time alleles at these multiple genetic loci, in addition to having the homozygous *PRNP* genotype of codon 129 methionine. Therefore, a human BSE epidemic may be multiphasic, and recent estimates of the size of the vCJD epidemic based on uniform genetic susceptibility could be substantial underestimations.[33,34] Genes implicated in species-barrier effects, which would further increase both the mean and range of human BSE incubation periods, are also probably relevant. In this context, a human epidemic will be difficult to accurately model until such modifier loci are identified and their gene frequencies in the population can be measured.[4]

Heterozygosity at *PRNP* codon 129 is a major determinant of susceptibility to and incubation time of human prion diseases.[5,7,9,35] As expected, most of these recent kuru cases with extended incubation periods (eight of ten) were heterozygotes. We have reported previously that most elderly survivors of exposure to traditional mortuary feasts are heterozygous.[9] Although the study included a small number of patients with kuru with long incubation periods, we saw no evidence of association with *PRNP* haplotype,[10] HLA-DQ7,[36] *APOE*,[36] or *PRND* alleles.[13]

Contributors

J Whitfield led the field patrol team throughout the study and investigated all suspect cases; E McKintosh provided assistance during this time. J Beck and S Mead undertook the molecular genetic studies. J Collinge, M P Alpers, E McKintosh, and D J Thomas did field neurological examinations. J Collinge and M P Alpers supervised the study and drafted the manuscript. All authors contributed to and approved the final version of the manuscript.

Conflict of interest statement

We declare that we have no conflict of interest.

Acknowledgments

This study would not have been possible without the generous support of the Fore communities and the assistance of many people in the UK and Papua New Guinea. We thank our team of local kuru reporters including Auyana Winagaiya, Anua Senagaiya, Igana Aresagu, Kabina Yaraki, Anderson Puwa, David Pako, Henry Pako, Pibi Auyana, Jolam Ove, Jack Kosinto, Dasta Hutu, Oma Aniimo, James Kisava, Sena Anua, David Ikabala, and Amalasa Pallar; and James Uphill, Mark Poulter, Tracy Campbell, Gary Adamson, and Huda Al-Doujaily for assistance with genetic analysis. We also thank John Millan, Peter Lantos, Chris Foster, Jonathan Boreham, Mike Devine, Anthony Jackson, Edward Lagan, and Lionel Astwood; John Reeder, Charles Mgone, and other staff at the Papua New Guinea Institute of Medical Research; Mark and Deborah Brandt of Open Bible Mission; and the British High Commission in Port Moresby for their help. This study was initially funded by a Wellcome Trust Principal Research Fellowship in the Clinical Sciences to JC, and since 2001 by the Medical Research Council. EM is a Wellcome Trust Clinical Training Fellow.

References

1 Collinge J. Prion diseases of humans and animals: their causes and molecular basis. *Annu Rev Neurosci* 2001; 24: 519–50.
2 Donnelly CA, Ferguson NM, Ghani AC, Anderson RM. Implications of BSE infection screening data for the scale of the British BSE epidemic and current European infection levels. *Proc R Soc Lond B Biol Sci* 2002; 269: 2179–90.
3 Ghani AC, Donnelly CA, Ferguson NM, Anderson RM. Updated projections of future vCJD deaths in the UK. *BMC Infect Dis* 2003; 3: 4.
4 Lloyd SE, Onwuazor ON, Beck JA, et al. Identification of multiple quantitative trait loci linked to prion disease incubation period in mice. *Proc Natl Acad Sci USA* 2001; 98: 6279–83.
5 Collinge J. Molecular neurology of prion disease. *J Neurol Neurosurg Psychiatry* 2005; 76: 906–19.
6 Alpers MP. Epidemiology and clinical aspects of kuru. In: Prusiner SB, McKinley MP, eds. Prions: novel infectious pathogens causing scrapie and Creutzfeldt-Jakob disease. San Diego, CA, USA: Academic Press, 1987: 451–65.
7 Palmer MS, Dryden AJ, Hughes JT, Collinge J. Homozygous prion protein genotype predisposes to sporadic Creutzfeldt-Jakob disease. *Nature* 1991; 352: 340–42.
8 Lee HS, Brown P, Cervenáková L, et al. Increased susceptibility to Kuru of carriers of the *PRNP* 129 methionine/methionine genotype. *J Infect Dis* 2001; 183: 192–96.
9 Mead S, Stumpf MP, Whitfield J, et al. Balancing selection at the prion protein gene consistent with prehistoric kurulike epidemics. *Science* 2003; 300: 640–43.
10 Mead S, Mahal SP, Beck J, et al. Sporadic—but not variant—Creutzfeldt-Jakob disease is associated with polymorphisms upstream of *PRNP* exon 1. *Am J Hum Genet* 2001; 69: 1225–35.
11 Alpers M. Epidemiological changes in kuru, 1957 to 1963. NINDB monograph no 2. Slow, latent and temperate virus infections. Bethesda, MD, USA: National Institute of Neurological Diseases and Blindness, 1965: 65–82.
12 Alpers MP. Kuru: implications of its transmissibility for the interpretation of its changing epidemiologic pattern. In: Bailey OT, Smith DE, eds. The central nervous system. some experimental models of neurological diseases. Baltimore: Williams & Wilkins, 1968: 234–51.
13 Mead S, Beck J, Dickinson A, Fisher EMC, Collinge J. Examination of the human prion protein-like gene *Doppel* for genetic susceptibility to sporadic and variant Creutzfeldt-Jakob disease. *Neurosci Lett* 2000; 290: 117–20.
14 Peoc'h K, Guerin C, Brandel JP, Launay JM, Laplanche JL. First report of polymorphisms in the prion-like protein gene (PRND): implications for human prion diseases. *Neurosci Lett* 2000; 286: 144–48.
15 Schröder B, Franz B, Hempfling P, et al. Polymorphisms within the prion-like protein gene (*Prnd*) and their implications in human prion diseases, Alzheimer's disease and other neurological disorders. *Hum Genet* 2001; 109: 319–25.
16 Chapman J, Cervenakova L, Petersen RB, et al. APOE in non-Alzheimer amyloidoses: transmissible spongiform encephalopathies. *Neurology* 1998; 51: 548–53.

17 Amouyel P, Vidal O, Launay JM, Laplanche JL. The apolipoprotein E alleles as major susceptibility factors for Creutzfeldt-Jakob disease. *Lancet* 1994; 344: 1315–18.

18 Jackson GS, Beck JA, Navarrete C, et al. *HLA-DQ7* antigen and resistance to variant CJD. *Nature* 2001; 414: 269–70.

19 Pepys MB, Bybee A, Booth DR, et al. MHC typing in variant Creutzfeldt-Jakob disease. *Lancet* 2003; 361: 487–89.

20 Hadlow WJ. Scrapie and kuru. *Lancet* 1959; 274: 289–90.

21 Gajdusek DC, Gibbs CJ Jr, Alpers MP. Experimental transmission of a Kuru-like syndrome to chimpanzees. *Nature* 1966; 209: 794–96.

22 Gajdusek DC. Unconventional viruses and the origin and disappearance of kuru. *Science* 1977; 197: 943–60.

23 Glasse R. Cannibalism in the Kuru region of New Guinea. *Trans N Y Acad Sci* 1967; 29: 748–54.

24 Lindenbaum S. Kuru sorcery: disease and danger in the New Guinea Highlands. Palo Alto, CA, USA: Mayfield, 1979.

25 Brown P, Gajdusek DC. Survival of scrapie virus after 3 years' interment. *Lancet* 1991; 337: 269–70.

26 Klitzman RL, Alpers MP, Gajdusek DC. The natural incubation period of kuru and the episodes of transmission in three clusters of patients. *Neuroepidemiology* 1984; 3: 3–20.

27 Collinge J. Variant Creutzfeldt-Jakob disease. *Lancet* 1999; 354: 317–23.

28 Brown P, Preece M, Brandel JP, et al. Iatrogenic Creutzfeldt-Jakob disease at the millennium. *Neurology* 2000; 55: 1075–81.

29 Barlow RM, Middleton DJ. Dietary transmission of bovine spongiform encephalopathy to mice. *Vet Rec* 1990; 126: 111–12.

30 Wells GAH, Hawkins SAC, Green RB, et al. Preliminary observations on the pathogenesis of experimental bovine spongiform encephalopathy (BSE): an update. *Vet Rec* 1998; 142: 103–06.

31 Lloyd SE, Uphill JB, Targonski PV, Fisher EM, Collinge J. Identification of genetic loci affecting mouse-adapted bovine spongiform encephalopathy incubation time in mice. *Neurogenetics* 2002; 4: 77–81.

32 Stephenson DA, Chiotti K, Ebeling C, et al. Quantitative trait loci affecting prion incubation time in mice. *Genomics* 2000; 69: 47–53.

33 Ghani AC, Ferguson NM, Donnelly CA, Anderson RM. Predicted vCJD mortality in Great Britain. *Nature* 2000; 406: 583–84.

34 D'Aignaux JNH, Cousens SN, Smith PG. Predictability of the UK variant Creutzfeldt-Jakob disease epidemic. *Science* 2001; 294: 1729–31.

35 Collinge J, Palmer MS, Dryden AJ. Genetic predisposition to iatrogenic Creutzfeldt-Jakob disease. *Lancet* 1991; 337: 1441–42.

36 Schachter F, Faure-Delanef L, Guenot F, et al. Genetic associations with human longevity at the *APOE* and *ACE* loci. *Nat Genet* 1994; 6: 29–32.

No. 16

医薬品 研究報告 調査報告書

識別番号・報告回数			報告日	第一報入手日	新医薬品等の区分	機構処理欄
				2006. 7. 7	該当なし	

一般的名称	(製造承認書に記載なし)	研究報告の公表状況	Saa P, Castilla J, Soto C. Science. 2006 Jul 7;313(5783):92-4.	公表国
販売名(企業名)	合成血「日赤」(日本赤十字社) 照射合成血「日赤」(日本赤十字社)			米国

研究報告の概要

○血中におけるプリオンの発症前検出

プリオンは伝達性海綿状脳症の病因となるタンパク様の感染性物質であると考えられている。PrP^{Sc}は、疾患の唯一の確認された代理マーカーであり、その検出は感染の拡大を最小限に抑えるために不可欠である。スクレイピーに感染したハムスターの血中から、疾患の発症前の期間の大半で、生化学的にPrP^{Sc}を検出した。潜伏期間の早期には、PrP^{Sc}はプリオンの末梢複製によって産生される傾向が強かった。一方、発症期には血液中のPrP^{Sc}は脳から漏出していた。感染したものの臨床症状を呈していない動物の血液中のプリオンの生化学的検出が可能になることで、TSEの早期の非侵襲的診断が見込まれる。

使用上の注意記載状況・その他参考事項等

合成血「日赤」
照射合成血「日赤」

血液を介するウイルス、細菌、原虫等の感染
vCJD等の伝播のリスク

報告企業の意見	今後の対応
スクレイピーに感染したハムスターの血中から、疾患の発症前の期間の大半で、生化学的にPrP^{Sc}を検出したとの報告である。	今後も引き続き、プリオン病に関する新たな知見及び情報の収集に努める。

8. A. Dagkessamanskaia *et al.*, *Mol. Microbiol.* 51, 1071 (2004).
9. S. Peterson *et al.*, *Mol. Microbiol.* 51, 1051 (2004).
10. I. Mortier-Barrière, A. de Saizieu, J. P. Claverys, B. Martin, *Mol. Microbiol.* 27, 159 (1998).
11. E. V. Pestova, L. S. Håvarstein, D. A. Morrison, *Mol. Microbiol.* 21, 853 (1996).
12. P. Luo, D. A. Morrison, *J. Bacteriol.* 185, 349 (2003).
13. B. Martin, M. Prudhomme, G. Alloing, C. Granadel, J. P. Claverys, *Mol. Microbiol.* 38, 867 (2000).
14. To monitor antibiotic-induced competence, cells were incubated in C+Y medium with an initial pH value adjusted so that spontaneous competence induction remained a rare event (see supporting online material).
15. D. E. Grove, S. Willcox, J. D. Griffith, F. R. Bryant, *J. Biol. Chem.* 280, 11067 (2005).
16. S. C. Kowalczykowski, *Annu. Rev. Biophys. Biophys. Chem.* 20, 539 (1991).
17. J. W. Little, *Proc. Natl. Acad. Sci. U.S.A.* 81, 1375 (1984).
18. B. Martin, P. García, M. P. Castanié, J. P. Claverys, *Mol. Microbiol.* 15, 367 (1995).
19. J. W. Beaber, B. Hochhut, M. K. Waldor, *Nature* 427, 72 (2004).
20. I. Phillips, E. Culebras, F. Moreno, F. Baquero, *J. Antimicrob. Chemother.* 20, 631 (1987).
21. C. Miller *et al.*, *Science* 305, 1629 (2004).
22. C. Levine, H. Hiasa, K. J. Marians, *Biochim. Biophys. Acta* 1400, 29 (1998).
23. R. A. VanBogelen, F. C. Neidhardt, *Proc. Natl. Acad. Sci. U.S.A.* 87, 5589 (1990).
24. K. Drlica, X. Zhao, *Microbiol. Mol. Biol. Rev.* 61, 377 (1997).
25. M. Cashel, D. R. Gentry, V. J. Hernandez, D. Vinella, in *Escherichia coli and Salmonella typhimurium: Cellular and Molecular Biology*, F. C. Neidhardt, Ed. (American Society for Microbiology Press, Washington, DC, 1996), pp. 1458–1496.

26. We thank D. Lane for critical reading of the manuscript. This work was supported in part by European Union grant QLK2-CT-2000-00543.

Supporting Online Material

www.sciencemag.org/cgi/content/full/313/5783/89/DC1
Materials and Methods
Figs. S1 to S4
Tables S1 and S2
References

27 March 2006; accepted 1 June 2006
10.1126/science.1127912

Presymptomatic Detection of Prions in Blood

Paula Saá,[1,2] Joaquín Castilla,[1] Claudio Soto[1]*

Prions are thought to be the proteinaceous infectious agents responsible for transmissible spongiform encephalopathies (TSEs). PrP^{Sc}, the main component of the infectious agent, is also the only validated surrogate marker for the disease, and its sensitive detection is critical for minimizing the spread of the disease. We detected PrP^{Sc} biochemically in the blood of hamsters infected with scrapie during most of the presymptomatic phase of the disease. At early stages of the incubation period, PrP^{Sc} detected in blood was likely to be from the peripheral replication of prions, whereas at the symptomatic phase, PrP^{Sc} in blood was more likely to have leaked from the brain. The ability to detect prions biochemically in the blood of infected but not clinically sick animals offers a great promise for the noninvasive early diagnosis of TSEs.

Prion diseases, also called transmissible spongiform encephalopathies (TSEs), are a group of fatal and infectious neurodegenerative diseases, including Creutzfeldt-Jakob disease (CJD) in humans and bovine spongiform encephalopathy (BSE), scrapie, and chronic wasting disease (CWD) in animals. Prions are composed mainly or exclusively of the misfolded prion protein (PrP^{Sc}) (*1*), which replicates in the body, transforming the normal prion protein (PrP^{C}) into more of the misfolded isoform.

Although prion diseases are rare in humans, the established link between a new variant form of CJD (vCJD) and BSE (*2*–*4*) has raised concern about a potential epidemic in the human population. Over the past few years, BSE has become a substantial health problem affecting many countries (*5*), and it seems now apparent that vCJD can be iatrogenically transmitted from human to human by blood transfusion (*6*, *7*). Exacerbating this state of affairs is the lack of a reliable test to identify individuals incubating the disease during the long and silent period from the onset of infection to the appearance of clinical symptoms (*8*–*10*).

PrP^{Sc} is not only the main component of the infectious agent and the most likely cause of the disease, but it is also the only validated surrogate marker for TSEs (*9*). However, PrP^{Sc} concentration is high enough for routine biochemical detection only in the brain and some lymphoid tissues at a time close to the symptomatic stage of the disease (*9*). The development of highly sensitive presymptomatic assays for the biochemical detection of PrP^{Sc} is critical for minimizing the spread of the disease (*9*). One important aim in prion diagnosis is the noninvasive and presymptomatic biochemical detection of PrP^{Sc} in biological fluids, particularly using blood, a fluid known to contain infectivity even before the onset of clinical signs (*6*, *11*, *12*).

PrP^{Sc} has been detected in the blood of sick animals by means of the protein misfolding cyclic amplification (PMCA) technology (*13*). PMCA produces accelerated prion replication, which dramatically amplifies the quantity of PrP^{Sc} present in a sample (*14*, *15*). In a cyclical process, large quantities of PrP^{C} are converted into the misfolded form triggered by the presence of minute and otherwise undetectable amounts of PrP^{Sc}. The method is highly specific for the detection of PrP^{Sc} and leads to a several-million-fold increase in sensitivity as compared to that of standard Western blot assays (*13*).

In order to evaluate the application of PMCA for the detection of prions in blood during the presymptomatic phase, 46 hamsters were inoculated intraperitoneally with 10% brain homogenate of the 263K scrapie strain, and 38 control animals were injected with phosphate-buffered saline (PBS). At different times during the incubation period, groups of animals were killed, blood was collected, and the buffy coat fraction was separated (*13*). Samples of the buffy coat were resuspended directly on healthy hamster brain homogenate and subjected to 144 PMCA cycles. Three different aliquots were tested from each sample. To refresh the substrate, after a round of PMCA cycling, samples were diluted 10-fold into normal brain homogenate, followed by another round of 144 PMCA cycles. This procedure was repeated seven times, because according to our results, this enables the detection of 20 to 50 molecules of monomeric hamster PrP, which seems to correspond to a single unit of infectious oligomeric PrP^{Sc} (*16*).

The first group of hamsters was killed 2 weeks after intraperitoneal inoculation. None of the five infected or control animals showed any detectable quantity of PrP^{Sc} in their blood (Fig. 1 and Table 1). Thus, the PrP^{Sc} present in the inoculum disappeared to undetectable levels during the first few days after inoculation. PrP^{Sc} was, however, readily detectable in blood 1 week later (20 days after inoculation) in 50% of the animals infected but in none of the controls (Fig. 1 and Table 1). The highest percentage of positive animals during the presymptomatic phase was observed 40 days after intraperitoneal inoculation, in which the sensitivity of PrP^{Sc} detection was 60%. After 60 days, the detection of PrP^{Sc} in blood became harder. Indeed, only one out of five animals scored positive at 70 days, whereas none of the five infected hamsters had detectable PrP^{Sc} in their blood 80 days after inoculation (Table 1). At the symptomatic stage, which in this experiment was at 114.2 ± 5.6 days, 80% of animals had PrP^{Sc} in their blood (Fig. 1). We never detected a false positive result in any of the 38 control samples analyzed (Table 1).

The distribution of PrP^{Sc} detection at different times of the incubation period showed

[1]George and Cynthia Mitchell Center for Alzheimer's Disease Research, Departments of Neurology, Neuroscience and Cell Biology, and Biochemistry and Molecular Biology, University of Texas Medical Branch, 301 University Boulevard, Galveston, TX 77555–0646, USA. [2]Centro de Biología Molecular, Universidad Autónoma de Madrid, Madrid, Spain.

*To whom correspondence should be addressed. E-mail: dsoto@utmb.edu

an interesting trend (Fig. 2). A first peak of PrP^{Sc} detection was observed early during the presymptomatic phase, between 20 and 60 days after inoculation. The peripheral administration of prions is known to result in an early phase of replication in lymphoid tissues and the spleen, before any infectious material reaches the brain (*17*, *18*). Indeed, little or no infectivity can be detected in the brain of animals peripherally inoculated during the first half of the incubation period (*19*). Thus, it is likely that the source of PrP^{Sc} in blood during the early presymptomatic phase is the spleen and other lymphoid organs. The quantity of PrP^{Sc} in blood goes down after this initial phase and actually disappears 80 days after inoculation (Table 1 and Fig. 2). The rise of PrP^{Sc} in blood during the early presymptomatic phase appears to coincide with the time of its exponential replication in lymphoid organs, whereas the reduction of PrP^{Sc} in blood occurs when infectivity in peripheral tissues has reached a plateau and is migrating from the periphery to the brain (*17*, *18*). Although the explanation for these results in unknown, it is possible that the proportion of circulating lymphocytes carrying PrP^{Sc} is much higher during the exponential phase of peripheral replication than during the stationary phase. At the symptomatic period, PrP^{Sc} can again be detected in the blood of most of the animals (Fig. 2). It has been reported that large quantities of PrP^{Sc} appear in the brain only a few weeks before the onset of clinical signs (*19*, *20*). Thus, PrP^{Sc} in blood samples at the symptomatic stage is likely to have come from brain leakage. It is known that at the time of symptomatic disease, TSE-affected individuals have extensive brain degeneration in the form of massive neuronal death, synaptic alterations, and brain inflammation (*21*). These abnormalities probably cause a disruption of the blood/brain barrier resulting in the leakage of cerebral proteins to the blood (*22*), in particular PrP^{Sc}, which by this time is highly abundant in the brain.

Infectivity studies have shown that the blood carries prions in both the symptomatic and presymptomatic stages of the disease in animals (*11*, *23*, *24*). Upon experimental BSE infection of sheep, infectivity can be transmitted by blood transfusion from asymptomatic infected animals (*25*), indicating that the infectious agent is present in blood during the incubation period. Recently, three cases of vCJD have been associated with blood transfusion from asymptomatic donors who subsequently died from vCJD (*6*, *7*). The alarmingly high proportion of cases transmitted by blood transfusion suggests that prions exist in relatively elevated quantities in the blood of individuals silently incubating vCJD. Based on studies with animal models, it is believed that all of the human population may be susceptible to vCJD infection (*26*), although clinical cases have so far occurred only in methionine homozygotes at codon 129 in the human prion protein gene. Because the incubation period may be several decades, it is currently unknown how many people may be in an asymptomatic phase of

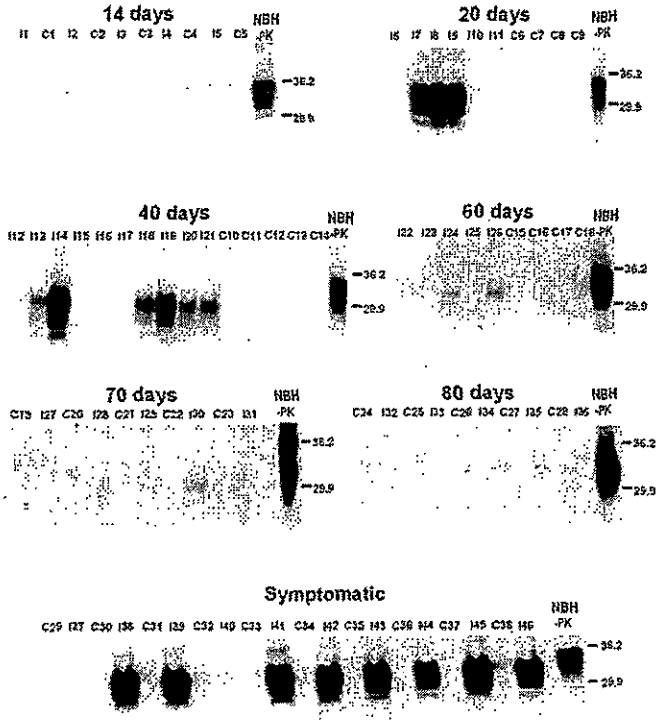


Fig. 1. PrP^{Sc} detection in the blood of scrapie-infected hamsters by PMCA. Blood samples from groups of scrapie-inoculated and control animals were taken at different times during the incubation period. Three milliliters of blood were separated in three aliquots of 1 ml each to prepare the buffy coat (*13*). Samples were subjected to 144 cycles of PMCA. Ten microliters of the sample from this first round of amplification were diluted into 90 μl of normal brain homogenate, and a new round of 144 PMCA cycles was performed. This process was repeated a total of seven times. Each panel represents the results obtained in the seventh round of PMCA with the samples from each group of animals, which are representative of the three independent aliquots taken from each animal. Ix, samples from hamsters infected with 263K scrapie; Cx, samples from control animals injected with PBS. All samples were treated with proteinase K (PK) before electrophoresis, except for the normal brain homogenate (NBH), in which no PK treatment (–PK) is indicated.

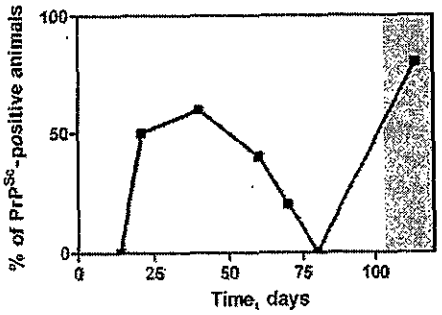


Fig. 2. Proportion of animals whose blood was PrP^{Sc} positive at different times during the incubation period. The percentage of samples scoring positive for PrP^{Sc} in blood is represented versus the time after inoculation at which samples were taken. Two phases of PrP^{Sc} detectability were observed: an early stage during the incubation period, which probably corresponds to the time during which peripheral prion replication in lymphoid tissues is occurring, and a second phase at the symptomatic stage, in which the brain contains extensive quantities of PrP^{Sc}. The vertical gray section indicates the symptomatic phase.

Table 1. Number of animals used and results obtained regarding the presymptomatic detection of PrP^{Sc} in the blood.

Time (days)	Controls (positives/total)	Infected (positives/total)	Sensitivity/ specificity
14	0/5	0/5	0%/100%
20	0/4	3/6	50%/100%
40	0/5	6/10	60%/100%
60	0/4	2/5	40%/100%
70	0/5	1/5	20%/100%
80	0/5	0/5	0%/100%
Symptomatic phase	0/10	8/10	80%/100%

vCJD infection. In addition, it is possible that some infected patients may never develop clinical symptoms but will remain asymptomatic carriers who can potentially transmit the disease to other individuals (*26*, *27*). In the absence of screening tests and effective therapies to treat this disease, a formidable worldwide public health challenge lies ahead to prevent further infections, assess infection rates, and treat infected patients. The ability to detect PrP^{Sc}, the major component of infectious prions, biochemically in the blood of infected but asymptomatic experimental animals will hopefully lead to the development of tests for human blood. Indeed, although technically more challenging, the PMCA technology has been adapted to amplify prions of human origin (*20*). The ability to accurately detect PrP^{Sc} in the presymptomatic stages of vCJD would potentially help to reduce the risk that many more people will be infected by this fatal and terrible disease.

References and Notes

1. S. B. Prusiner, *Proc. Natl. Acad. Sci. U.S.A* 95, 13363 (1998).
2. S. N. Cousens, E. Vynnycky, M. Zeidler, R. G. Will, R. G. Smith, *Nature* 385, 197 (1997).
3. J. Collinge, *Lancet* 354, 317 (1999).
4. M. E. Bruce *et al.*, *Nature* 389, 498 (1997).
5. R. Bradley, P. P. Liberski, *Folia Neuropathol.* 42 (suppl. A), 55 (2004).
6. C. A. Llewelyn *et al.*, *Lancet* 363, 417 (2004).
7. A. H. Peden, M. W. Head, D. L. Ritchie, J. E. Bell, J. W. Ironside, *Lancet* 364, 527 (2004).
8. Q. Schiermeier, *Nature* 409, 658 (2001).
9. C. Soto, *Nat. Rev. Microbiol.* 2, 809 (2004).
10. L. Ingrosso, V. Vetrugno, F. Cardone, M. Pocchiari, *Trends Mol. Med.* 8, 273 (2002).
11. P. Brown, L. Cervenakova, H. Diringer, *J. Lab. Clin. Med.* 137, 5 (2001).
12. F. Houston, J. D. Foster, A. Chong, N. Hunter, C. J. Bostock, *Lancet* 356, 999 (2000).
13. J. Castilla, P. Saa, C. Soto, *Nat. Med.* 11, 982 (2005).
14. G. P. Saborio, B. Permanne, C. Soto, *Nature* 411, 810 (2001).
15. C. Soto, G. P. Saborio, L. Anderes, *Trends Neurosci.* 25, 390 (2002).
16. J. R. Silveira *et al.*, *Nature* 437, 257 (2005).
17. R. H. Kimberlin, C. A. Walker, *J. Comp. Pathol.* 89, 551 (1979).
18. M. Glatzel, A. Aguzzi, *Microbes Infect.* 2, 613 (2000).
19. R. H. Kimberlin, C. A. Walker, *J. Gen. Virol.* 67, 255 (1986).
20. C. Soto *et al.*, *FEBS Lett.* 579, 638 (2005).
21. J. Castilla, C. Hetz, C. Soto, *Curr. Mol. Med.* 4, 397 (2004).
22. W. A. Banks, *J. Neurovirol.* 5, 538 (1999).
23. P. Brown, *Vox Sang.* 89, 63 (2005).
24. N. Hunter *et al.*, *J. Gen. Virol.* 83, 2897 (2002).
25. N. Hunter, *Br. Med. Bull.* 66, 171 (2003).
26. M. T. Bishop *et al.*, *Lancet Neurol.* 5, 393 (2006).
27. J. W. Ironside, *Haemophilia* 12, 8 (2006).

28. This research was supported in part by NIH grants AG0224642 and NS049173.

Supporting Online Material

www.sciencemag.org/cgi/content/full/313/5783/92/DC1
Materials and Methods
References

21 April 2006; accepted 5 June 2006
10.1126/science.1129051

Prion-Induced Amyloid Heart Disease with High Blood Infectivity in Transgenic Mice

Matthew J. Trifilo,[1] Toshitaka Yajima,[2] Yusu Gu,[2] Nancy Dalton,[2] Kirk L. Peterson,[2] Richard E. Race,[3] Kimberly Meade-White,[3] John L. Portis,[3] Eliezer Masliah,[4] Kirk U. Knowlton,[2]* Bruce Chesebro,[3]* Michael B. A. Oldstone[1]*

We investigated extraneural manifestations in scrapie-infected transgenic mice expressing prion protein lacking the glycophosphatydylinositol membrane anchor. In the brain, blood, and heart, both abnormal protease-resistant prion protein (PrPres) and prion infectivity were readily detected by immunoblot and by inoculation into nontransgenic recipients. The titer of infectious scrapie in blood plasma exceeded 10^7 50% infectious doses per milliliter. The hearts of these transgenic mice contained PrPres-positive amyloid deposits that led to myocardial stiffness and cardiac disease.

In humans and animals, transmissible spongiform encephalopathies (TSEs), or prion diseases, cause neurodegeneration and death following ingestion or experimental inoculation of infected material. Prion diseases are characterized by the conversion of the normal protease-sensitive host prion protein (PrPsen) to a disease-associated protease-resistant form (PrPres). Although prion disease damages the central nervous system (CNS), infectivity and PrPres can be detected within peripheral tissues, including lymphoid organs in humans, sheep, and deer (*1*, *2*), as well as skeletal muscle (*3*), kidney, and pancreas (*4*) of some transgenic rodent models. Despite the toxic effect on the CNS, few if any histopathological changes have been observed at peripheral sites.

Transmission of TSE disease to humans has resulted from cannibalism, contaminated surgical instrumentation, and tainted growth hormone (*5*–*7*). A human disease termed variant Creutzfeldt-Jakob disease (vCJD) has occurred more recently, apparently through the ingestion of bovine spongiform encephalopathy (BSE)–infected cattle products (*8*). Recent evidence suggests that transmission of vCJD between humans may occur through blood transfusion (*9*, *10*), and this conclusion is supported by experimental transmission of BSE between sheep via blood transfusion (*11*). TSE infectivity has been demonstrated in blood by intracerebral-inoculation in mouse, mink, hamster, and goat models (*7*, *12*–*20*). However, infectivity in such cases is low, $\leq 10^2$ 50% infectious doses (ID_{50}) per ml of blood compared to 10^6 to 10^{10} ID_{50}/g in the brain.

Normal prion protein, PrPsen, is expressed primarily as a membrane-bound, glycophosphatydylinositol (GPI)–anchored protein. The role of cellular PrP membrane anchoring in prion disease has been studied in transgenic mice expressing GPI-negative anchorless PrP, which is secreted from cells (*21*). Intracerebral inoculation of these GPI-negative anchorless PrP transgenic (tg) mice with murine scrapie results in scrapie replication and deposition of PrPres within the brain. Although wild-type (WT) mice infected with scrapie usually develop a nonamyloid form of PrPres, in these tg mice the PrPres is primarily in the form of amyloid plaques (*21*). At the same time, these mice do not manifest the clinical and pathologic alterations normally associated with prion disease, thus demonstrating a separation between PrPres amyloid accumulation and clinical CNS disease (*21*). In the brain of these infected tg mice, PrPres was located primarily within and around endothelial cells (*21*) (Fig. 1A), leading to the hypothesis that anchorless PrPres may be secreted in the blood. Here we examined this possibility.

To determine whether PrPres and/or scrapie infectivity was present in blood, four infected tg mice were bled between 450 and 512 days postinfection (dpi) with the RML strain of scrapie. Inoculation of a 1:500 dilution of blood from all four mice induced scrapie in WT (C57BL/6) recipients in ~145 days. In addition, blood of two mice analyzed by serial dilution titration gave titers of $\geq 1.6 \times 10^7$ and $\geq 1.6 \times 10^5$ ID_{50}/ml blood (Table 1).

[1]Viral-Immunobiology Laboratory, Departments of Molecular and Integrative Neurosciences and Infectology, Scripps Research Institute, La Jolla, CA 92037, USA. [2]Department of Medicine, University of California, San Diego, La Jolla, CA 92093, USA. [3]Laboratory of Persistent Viral Diseases, Rocky Mountain Laboratories, National Institute of Allergy and Infectious Diseases, Hamilton, MT 59840, USA. [4]Departments of Neurosciences and Pathology, University of California, San Diego, CA 92093, USA.

*To whom correspondence should be addressed. E-mail: mbaobo@scripps.edu (M.B.A.O.), bchesebro@niaid.nih.gov (B.C.), kknowlton@ucsd.edu (K.U.K.)

別紙様式第2-1

No. 15

医薬品 研究報告 調査報告書

識別番号・報告回数			報告日	第一報入手日	新医薬品等の区分	機構処理欄
				2006. 5. 11	該当なし	

一般的名称	(製造承認書に記載なし)	研究報告の公表状況	Guardian Unlimited, The Guardian. 2006 May 2.	公表国
販売名(企業名)	合成血「日赤」(日本赤十字社) 照射合成血「日赤」(日本赤十字社)			英国

研究報告の概要

○英国の血漿分画製剤が14カ国でvCJDのリスクを引き起こす可能性
英国政府は、1990年代に輸出された英国産の血漿分画製剤によって患者にvCJDのリスクがあると14カ国に警告した。
2003年12月に輸血によるvCJD感染が英国で発生し、血液製剤による感染伝播に対する安全対策が実施された。当局は、国営企業Bio Products Laboratory(BPL)から海外に輸出された製剤を再調査する必要に迫られた。
当局は、vCJDの潜在的リスクは「非常に不確実」であるとしながらも、ブラジルとトルコの保健省に対して予防措置を取るよう勧告した。この措置には患者の追跡調査、供血や臓器・組織提供をしないよう求めることが含まれる。また、患者は医師や歯科医師に治療を受ける際に申し出るよう求められる。
英国内では、危険のある人は最大で6000人と考えられる。問題は、血漿分画製剤が何千もの供血血液を処理して製造されることである。vCJDを発症した9人の供血者のたった23供血から懸念が生じた。
BPLを所管するNHSの血液・移植部門は「これまで血漿分画製剤が関係したvCJD症例はない。英国の血漿由来の製剤の使用は1999年に終了した。しかし、供血経験のある人がvCJDと診断されたため、終了以前に製剤を投与された患者にリスクがある可能性が出てきた。2004年以降、1980年以降に輸血を受けた人は供血不可としている」と話している。
ブルネイ、UAE、インド、ヨルダン、オマーン、シンガポールはブラジルやトルコに比べて危険性は低いものの、予防措置をとる必要がある。ベルギー、モロッコ、エジプト、フランス、オランダ、イスラエルには危険性の少ない製剤が輸出されており、製造の最終工程はこれらの国で行われたため、各国での分析を続けるよう勧告された。フランス政府は、同国から他の10カ国に再輸出された製剤には危険はないとしている。

報告企業の意見	今後の対応
英国政府は、英国産の汚染された血漿分画製剤によって患者にvCJDのリスクがあると14カ国に警告したとの報告である。	今後も引き続き、プリオン病に関する新たな知見及び情報の収集に努める。

使用上の注意記載状況・その他参考事項等

合成血「日赤」
照射合成血「日赤」

血液を介するウイルス、細菌、原虫等の感染
vCJD等の伝播のリスク

British blood products may pose vCJD risk in 14 countries

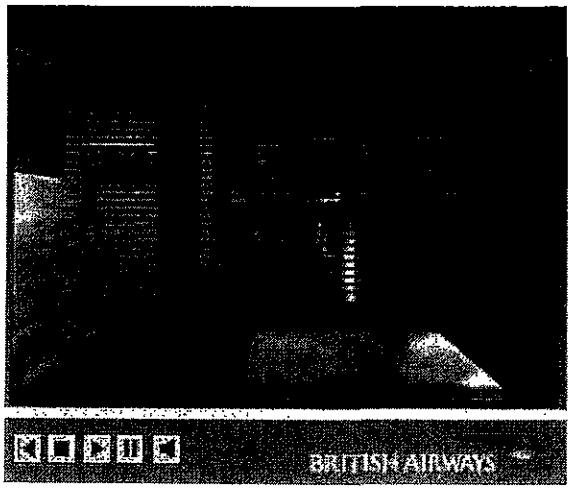
- UK issues warning on 'mad cow disease'
- Documents show Brazil and Turkey are high on list

James Meikle and Rob Evans
Tuesday May 2, 2006
The Guardian

The government has been forced to warn 14 countries that patients are in danger of developing the human form of mad cow disease as a result of contaminated British blood products sold abroad.

Documents released under the Freedom of Information Act show that patients in Brazil and Turkey are most at risk from the products, although it is too early to know how many, if any, foreign patients may develop the incurable variant CJD, as it takes many years to appear. The Turkish authorities said they had traced patients at risk and were closely monitoring them, while Brazil would not comment.

Article continues



The contaminated blood products were exported in the 1990s by the British government to treat conditions such as haemophilia, severe burns and immune deficiency. At the time the government considered there was no risk.

Twenty-eight people abroad have already developed vCJD by eating cattle meat from Britain infected with BSE. However, the

dangers of another route of transmission are now becoming more evident. Scientists are worried about a "second wave" of casualties caused by blood donated by people infected but not yet displaying symptoms of the disease.

The risk of passing on the disease in this way was considered only theoretical until December 2003, when it emerged that a patient in Britain had been infected through a blood transfusion, leading to new safety measures. Another two cases have since been identified. Health authorities then had to re-examine blood products sent abroad by the state-owned company Bio Products Laboratory (BPL).

The documents show that, following the rethink, the Health Protection Agency was concerned "about the potential infectivity of blood". Believing the potential risk of vCJD to be "very uncertain", the agency advised the Brazilian and Turkish health ministries to take precautions to reduce the possibility of spreading vCJD as "sufficient quantities" of the "at-risk" products had been exported.

These measures included tracking down patients and telling them not to donate blood, organs or tissues. Patients are also told to inform doctors and dentists if they need any treatment.

In Britain, up to 6,000 people were considered to be at risk. The problems stem from the way blood products are made, from processing thousands of separate donations. The concerns arise from just 23 donations made by nine people who went on to develop vCJD, showing how minute amounts may be infectious.

The NHS Blood and Transplant Authority, which is responsible for BPL, said: "So far no vCJD cases have been linked to plasma products ... The use of products derived from British blood plasma was ended in 1999 as a precautionary safety measure because of what were then regarded as only theoretical risks. But cases where patients might have been put at risk before that date have since come to light as further cases of vCJD have been diagnosed in people who were blood donors. Since 2004, no one who received a blood transfusion after 1980 has been allowed to donate blood themselves."

The Health Protection Agency decided that patients in six countries - Brunei, UAE , India, Jordan, Oman and Singapore - had been put in less jeopardy than those in Brazil and Turkey, but might need to take precautions. Less dangerous batches were imported by Belgium, Morocco and Egypt. France, Holland and Israel were advised to carry out their own assessments, as manufacture of the blood products was completed in their countries. The French government concluded that there was no danger from the products, which were re-exported to 10 unnamed countries.

The Guardian has previously reported that patients worldwide may have been exposed to vCJD, but the documents detail for the first time the countries, the amounts and the risk assessments. British authorities cannot say how many patients abroad may now be in danger.

There have been 161 cases of vCJD in Britain. There are 15 cases in France, four in Ireland, two in the US, and one each in Canada, Italy, Japan, the Netherlands, Portugal, Saudi Arabia and Spain.

Some of these victims are known to have caught vCJD by eating infected beef in Britain. Most others live in countries that

have also had outbreaks of BSE that may well have originated from Britain.

Graham Steel, whose brother Richard died from vCJD, drew parallels to the spread of BSE. "[It is] eerily reminiscent of the 1980s when 'theoretically' infectious meat and bonemeal was exported by the UK around Europe and beyond despite the fact that the risks of spreading diseases were known about in 1972-73. A total recall was deemed too expensive."

Special reports
BSE and CJD
What's wrong with our food?

Explained
The threat to humans from BSE

Cartoon
Steve Bell on the BSE report

Useful links
Official BSE inquiry
Human BSE Foundation
CJD Support Network
British Medical Journal CJD page
Department of Health CJD/BSE page
Department for Environment, Food and Rural Affairs BSE page
The UK CJD disease surveillance unit
Food Standards Agency BSE page

Printable version | Send it to a friend | Save story

Daily sections

Weekly sections

医薬品
医薬部外品　研究報告　調査報告書
化粧品

識別番号・報告回数		回	報告日 年　月　日	第一報入手日 2006 年 4 月 6 日	新医薬品等の区分 該当なし	総合機構処理欄
一般的名称			研究報告の公表状況	Preparation of soluble infectious samples from scrapie-infected brain: a new tool to study the clearance of transmissible spongiform encephalopathy agents during plasma fractionation Vito Angelo Berardi, et al. Lancet 2006; 46: 652- 8	公表国 イタリア	
販売名（企業名）						

研究報告の概要

英国で血液感染による変異型クロイツフェルト・ヤコブ病が 2 例報告されて以来，プリオンと関わる血液，血液成分，及び血漿由来製剤の安全性が大きく懸念されている。感染性海綿状脳症(TSE)に感染した齧歯類の脳の一部をスパイクした血液成分や血漿由来製剤からのプリオンの除去，あるいは不活性化を可能にする方法の開発が取り組まれた。しかしながら，スパイクには恐らく血液感染とは関連のない病原性プリオンタンパク質 (PrP^{TSE})の凝集体が含まれており，この集合体の存在により試験が無効になる可能性がある。これを明らかにするため，筆者らは以下の実験を行った。
スクレーピーのハムスター263K 株の脳組織を 10%リン酸緩衝食塩水で懸濁し，低速遠心分離後，上澄み液を収集し，30 分間２５ 220,000×g で高速遠心分離を行った。高速遠心による浮遊物 (S^{HS})と沈殿物を収集し，ウエスタンブロット法によりプロテイナーゼ耐性 PrP^{TSE}を，離乳ハムスターへ脳内接種し感染性を測定した。実験では，相当量のプリオン感染力(脳組織の 10%懸濁液の mL あたりの 50%致死量 105 以上)がスクレーピーのハムスター263K 株の S^{HS}分画でみられたが，その効果は PrP^{TSE}の凝集体を含む分画よりも低かった。一方，この分画はなにも含んでいないか，含んだとしても僅か微量の PrP^{TSE}の凝集体であることが確認された。本実験で示された PrP^{TSE}の凝集体を含まない感染性物質を調製する簡便な方法は，ヒト血液成分及び血漿由来製剤におけるプリオン除去，または不活化確認試験に用いるのに適当なスパイク物質を調製する上で有用であると考えられた。

使用上の注意記載状況・その他参考事項等

BYL-2006-0215

報告企業の意見	今後の対応
弊社の血漿分画製剤の製造工程において，2004 年の Position statement で欧州医薬品審査庁が推奨する，プリオン除去及び感染性評価の開発を行った。その結果，各製品の製造過程で，少なくとも 4log のプリオン除去が可能であることが確認されている。	現時点で新たな安全対策上の措置を講じる必要は無いと考える。引き続き関連情報の収集に努める。

Preparation of soluble infectious samples from scrapie-infected brain: a new tool to study the clearance of transmissible spongiform encephalopathy agents during plasma fractionation

Vito Angelo Berardi, Franco Cardone, Angelina Valanzano, Mei Lu, and Maurizio Pocchiari

BACKGROUND: Concern about the safety of blood, blood components, and plasma-derived products with respect to prions has increased since the report of two blood-related infections of variant Creutzfeldt-Jakob disease in the United Kingdom. Efforts were directed toward the development of procedures able to remove or inactivate prions from blood components or plasma-derived products with brain fractions of transmissible spongiform encephalopathy (TSE)-infected rodents as spiking materials. These spiking materials, however, are loaded with pathological prion protein (PrP^{TSE}) aggregates that are likely not associated to blood infectivity. The presence of these aggregates may invalidate these studies.

STUDY DESIGN AND METHODS: Brains from 263K scrapie–infected hamsters were suspended in 10 percent phosphate-buffered saline. After low-speed centrifugation, the supernatant was collected and ultracentrifuged at 220,000 × *g* at 25°C for 30 minutes. The high-speed supernatants (S^{HS}) and pellets were collected; the proteinase-resistant PrP^{TSE} was measured by Western blot and infectivity by intracerebral inoculation into weanling hamsters.

RESULTS: A substantial amount of prion infectivity (more than 10^5 LD_{50} per mL of a 10% suspension of brain tissues) is present in the S^{HS} fraction of 263K scrapie–infected hamster brains. Concomitantly, this fraction contains none or only traces of PrP^{TSE} in its aggregate form.

CONCLUSION: This study describes a simple and fast protocol to prepare infectious material from 263K scrapie–infected brains that is not contaminated with PrP^{TSE} aggregates. This S^{HS} fraction is likely to be the most relevant material for endogenous spiking of human blood in validation experiments aimed at demonstrating procedures to remove or inactivate TSE infectious agents.

The occurrence of two blood-related infections of variant Creutzfeldt-Jakob disease (vCJD) in the United Kingdom[1,2] and the finding that approximately 10 percent of vCJD cases were blood donors before the appearance of clinical signs[1] are cause of increasing concern for the safety of blood transfusion and, as a consequence, of blood components or plasma-derived products. There is strong evidence that vCJD is caused by the consumption of bovine spongiform encephalopathy (BSE)-contaminated meat products, but the occurrence of human-to-human transmission of vCJD has now raised the possibility that other cases might be related to blood transfusions rather than meat consumption. BSE and vCJD, together with scrapie in sheep and goats, sporadic and genetic CJD, Gerst-

ABBREVIATIONS: BSE = bovine spongiform encephalopathy; LD_{50} = doses required to kill 50 percent of inoculated animals; NaPTA = sodium phosphotungstic acid; PK = proteinase K; P^{HS} = high-speed pellet; P^{NaPTA} = pellet after sodium phosphotungstic acid precipitation; PrP^{TSE} = pathological prion protein; PrP^{27-30} = 27- to 30-kDa fragment of protease-resistant prion protein; S^{LS} = low-speed supernatant; S^{HS} = high-speed supernatant; TSE = transmissible spongiform encephalopathy; TBST = Tris-buffered saline (pH 8) with 0.05 percent Tween 20; vCJD = variant Creutzfeldt-Jakob disease.

From the Degenerative and Inflammatory Neurological Diseases Unit, Department of Cell Biology and Neurosciences, Italian National Institute of Health, Rome, Italy.

Address reprint requests to: Maurizio Pocchiari, MD, Degenerative and Inflammatory Neurological Diseases Unit, Department of Cell Biology and Neurosciences, Istituto Superiore di Sanità, Viale Regina Elena 299, 00161 Rome, Italy; e-mail: pocchia@iss.it.

Partially supported by the Ministero della Salute, Italy, Progetto Finalizzato 1% 2001/1ABR.

Received for publication July 8, 2005; revision received August 30, 2005, and accepted August 30, 2005.

doi: 10.1111/j.1537-2995.2006.00763.x

TRANSFUSION 2006;46:652-658.

mann-Sträussler-Scheinker disease, and sporadic or familial fatal insomnia, belong to the group of transmissible spongiform encephalopathy (TSE) or prion diseases that are progressive degenerative disorders of the central nervous system with fatal outcome.[3] The majority of vCJD cases have occurred in the United Kingdom (n = 159)[4] or in patients who spent months in the United Kingdom before the development of disease. However, vCJD has also occasionally been reported in patients who were not British and never traveled to the United Kingdom, suggesting that these patients were infected in their own country.[5-8] Moreover, a preliminary prospective study in the United Kingdom has indicated that there are about 3000 individuals in the age group 10 to 30 years who might carry prion infectivity in the lymphoreticular tissues[9] and, possibly, in the blood. The findings that vCJD patients carry infectivity in blood up to 3 years before the appearance of clinical signs[1] and that no test is yet available for the screening of vCJD-infected people[10,11] have focused the efforts for the safety of blood toward procedures that may remove or inactivate the infectious agent in blood, blood components, or plasma products. These validation experiments are usually performed either with blood taken from TSE-infected rodents or with human blood spiked with TSE-infected rodent brains.[12] Blood of TSE-infected rodents, however, contains only up to 10^2 infectious doses per mL,[13,14] and even the complete removal of these low levels of infectivity does not guarantee the efficacy of the treatment and the safety of blood.[12] Spiking blood with TSE-infected brains greatly increases the amount of infectivity and therefore overcomes the low-level infectivity naturally carried in rodent blood, but the criticism of these validation studies is that the nature of prions in the brain may substantially differ from that present in the blood.[12] Most of the infectivity in the brain is associated with the abnormal prion protein (PrP^{TSE}) in its aggregate form, whereas in blood it is likely that infectivity is associated to a much more soluble fraction of PrP^{TSE}. This substantial difference in the physicochemical structure of PrP^{TSE}-associated infectivity may influence the efficacy of procedures able to inactivate or remove prion infectivity.

In this article, we show that fractions of 263K scrapie–infected brains retain a high level of prion infectivity without being associated with the aggregate form of PrP^{TSE}. This fraction might be useful in the validation studies of pharmaceuticals products derived from blood or urine collected from human or BSE-susceptible ruminants.

MATERIALS AND METHODS

Extraction of water-soluble scrapie infectivity

263K scrapie–infected hamster brains were suspended in 9 vol of sterile phosphate-buffered saline (PBS; pH 7.4) and homogenized by use of a Teflon-glass Potter tissue grinder. The homogenate was dispersed with 10 sonication pulses (Vibra Cell, Sonics & Materials Inc., Newtown, CT) while kept on ice and then centrifuged at $825 \times g$ for 15 minutes at 25°C (GS-6R, rotor GH-3.7, Beckman Coulter, Fullerton, CA). Low-speed supernatant (S^{LS}) was sonicated as above and ultracentrifuged at $220{,}000 \times g$ for 30 minutes at 25°C (Optima TL-100, rotor TLA 100.3, Beckman Coulter, Fullerton, CA). This high-speed supernatant (S^{HS}) was collected and the high-speed pellet (P^{HS}) was sonicated in sterile PBS to obtain a 10 percent suspension (gram-equivalents of brain/PBS). In Replicate 2, sonication was never performed. These three fractions (S^{LS}, S^{HS}, and P^{HS}) were stored at –70°C until assayed. The S^{HS} fraction of Replicate 3 was examined by transmission electron microscopy after negative staining.

Western blot measurement of the 27- to 30-kDa fragment of protease-resistant prion protein

Fractions S^{LS}, S^{HS}, and P^{HS} were thawed and treated for 60 minutes at 37°C with proteinase K (PK; Sigma Chemical Co., St. Louis, MO) at a final enzyme concentration of 50 µg per mL. The digestion was stopped by adding protease inhibitors (Complete, Roche Diagnostics GmbH Roche Applied Science, Mannheim, Germany) in accordance with the manufacturer's instruction.

Sodium dodecyl sulfate–polyacrylamide gel electrophoreses and Western blot assays were performed according to Lee and coworkers[15] with some modifications. After PK treatment, the samples were serially diluted in half-log steps in NuPAGE gel loading buffer, boiled for 10 minutes in a water bath, and electrophoresed on 12 percent NuPAGE Bis-Tris gels (Invitrogen Corp., Carlsbad, CA) for 60 minutes at 125 V. The nitrocellulose membrane (Hybond ECL, Amersham Biosciences Europe GmbH, Freiburg, Germany) was soaked in Towbin transfer buffer for 5 minutes before "sandwich" assembly and semidry transfer for 60 minutes at 125 mA at 4°C. The membrane was blocked for 60 minutes at 37°C in 3 percent nonfat dry milk (Bio-Rad, Hercules, CA), dissolved in Tris-buffered saline (pH 8) with 0.05 percent Tween 20 (TBST), and incubated overnight at 4°C with 3F4 monoclonal anti-hamster 27- to 30-kDa fragment of protease-resistant prion protein ($PrP^{27\text{-}30}$) antibody[16] (provided by H. Diringer) diluted 1:2000 in TBST. The membrane was rinsed with TBST (five changes of solution in 25 min), incubated for 90 minutes at room temperature with an alkaline phosphatase–labeled goat anti-mouse IgG (Perkin-Elmer Sciences, Wellesley, MA) diluted at 1:5000 in TBST, and rinsed again. Bands were revealed by the CDP-star chemiluminescence detection kit (Applied Biosystems, Foster City, CA) and

recorded onto sensitive films (Hyperfilm ECL, Amersham Biosciences).

Sodium phosphotungstic acid precipitation of the S^{HS} fraction

To recover PrP^{TSE} in fraction S^{HS}, the sample was mixed with 1 volume of 4 percent sarkosyl and processed with sodium phosphotungstic acid (NaPTA; 0.3%) and $MgCl_2$ (12.75 mmol/L) as published by Wadsworth and associates[17] with the only modification consisting in the precipitation of the final pellet by ultracentrifuge at 220,000 × *g* for 30 minutes at 25°C (Optima TL-100, rotor TLA 100.3). The pellet (P^{NaPTA}) was then sonicated in sterile PBS to obtain a 10 percent suspension (gram-equivalents of brain/PBS) and stored at –70°C until assayed.

Infectivity bioassay

Groups of 7 to 10 Syrian hamsters were anesthetized and then inoculated intracerebrally with 50 µL of fractions S^{LS}, S^{HS}, P^{HS}, and P^{NaPTA}. Animals were maintained in coded plastic cages with water and food ad libitum and regularly scored for clinical signs of scrapie disease as previously described.[18] Incubation periods (mean ± SD) were measured and infectivity titers were estimated by applying these values to a dose incubation curve drawn after an endpoint titration.[19] An inverse relation exists between dose and incubation period in the 263K strain in hamsters, which gives a mean incubation period of 155.5 days for 1 LD_{50} intracerebral unit in 0.05 mL of a 10 percent brain homogenate.[19] Animals were housed at the animal facility of the Italian National Institute of Health (Istituto Superiore di Sanità, ISS) under the supervision of the Service for Biotechnology and Animal Welfare of the ISS who warrants the adherence to national and international regulations on animal welfare.

RESULTS

Western blot analyses of 263K scrapie–infected brain fractions

As expected, a great amount of partially PK-resistant PrP^{TSE} (PrP^{27-30} Fig. 1) and infectivity (approx. 8 log LD_{50}/mL 10% brain suspension) is present in the supernatant (S^{LS}) after low-speed centrifugation of 263K scrapie–infected brain homogenates in PBS. The majority of PrP^{27-30} and infectivity is then recovered in the pellet after ultracentrifugation (P^{HS}). As shown in Table 1, the difference

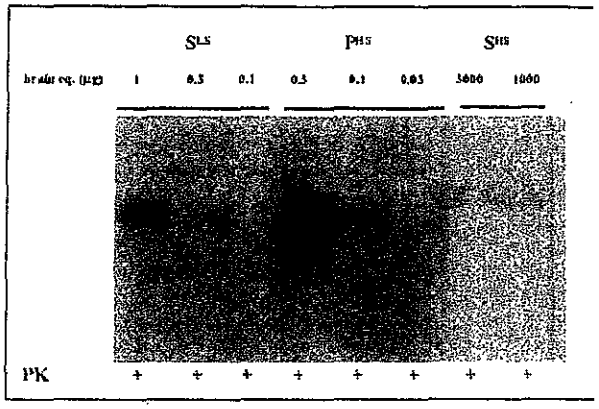


Fig. 1. Western blot analysis of PrP^{27-30} in low-speed (S^{LS}) and high-speed (P^{HS} and S^{HS}) fractions prepared from 263K scrapie–infected brain (Replicate 3). The samples were treated with PK, diluted in half-log steps in NuPAGE gel loading buffer, and resolved on a 12 percent NuPAGE Bis-Tris gels. After the transfer to nitrocellulose membrane and incubation with monoclonal antibody 3F4, PrP^{27-30} was visualized by chemiluminescence on sensitive films. PrP^{27-30} was not measurable in S^{HS}, indicating that virtually all the aggregate form of PrP^{TSE} was precipitated after centrifugation at 220,000 × *g* for 30 minutes.

TABLE 1. Endpoint titration of PrP^{27-30} in different fractions (S^{LS}, S^{HS}, and P^{HS}) of 263K scrapie–infected brain

Replicate number	Fraction	Log dilutions (weight-equivalents of brain tissue) 0.5 (3 mg)	1 (1 mg)	4 (1 µg)	4.5 (0.3 µg)	5 (0.1 µg)	5.5 (0.03 µg)	6 (0.01 µg)	Difference (log) between S^{LS} and S^{HS}
1	S^{LS}			+	+	–			≥4.5
	S^{HS}	–	–						
	P^{HS}			+	+	+	–		
2*	S^{LS}			+	–				≥4.0
	S^{HS}	–	–						
	P^{HS}			+	–				
3	S^{LS}			+	+	+	–		≥5.0
	S^{HS}	–	–						
	P^{HS}			+	+	+	+	–	
4	S^{LS}			+	+	+	–		4.5
	S^{HS}	+	–						
	S^{NaPTA}†	–							
	P^{NaPTA}	+	–						

* No sonication was performed in this replicate.
† S^{NaPTA} = supernatant after sodium phosphotungstic acid precipitation.

between the amount of PrP^{27-30} in S^{LS} and P^{HS} was either null (Replicate 2) or no more than 0.5 log (Replicates 1 and 3) and it was not influenced by the use of sonication for the dispersion of samples (compare Replicates 1 and 3 with Replicate 2). Concordantly, PrP^{27-30} was either not measurable (Fig. 1, Replicate 3) or present at a very low amount (Fig. 2, Replicate 4) in the supernatant after ultracentrifuge (S^{HS}, Table 1), indicating that virtually all the aggregate form of PrP^{TSE} was precipitated by 220,000 × *g* for 30 minutes. In other words, the ultracentrifuge reduces the amount of PrP^{TSE} aggregates in the supernatant of more than 10,000 times (Table 1). Examination of the S^{HS} fraction of Replicate 3 revealed amorphous proteinaceous material and no delimiting membranous structure. In Replicate 4, the small amount of PrP^{TSE} was completely precipitated by NaPTA precipitation (P^{NaPTA}; Fig. 2). In the other replicates, PrP^{TSE} was not recovered even after NaPTA precipitation.

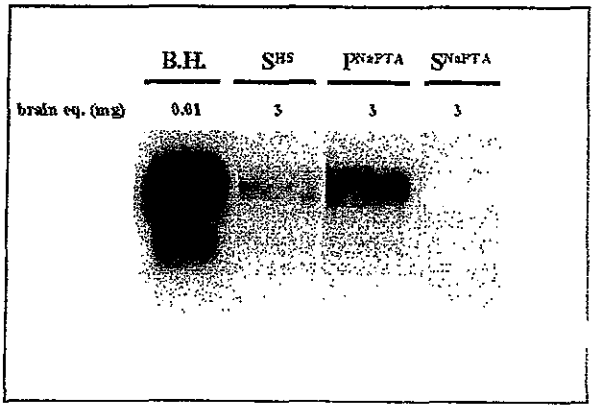


Fig. 2. Western blot analysis of PrP^{27-30} in S^{HS} fraction before (S^{HS}) and after (S^{NaPTA}) NaPTA precipitation (Replicate 4). The samples were treated with PK, resolved on a 12 percent NuPAGE Bis-Tris gels, and transferred to nitrocellulose membrane. After incubation with monoclonal antibody 3F4, PrP^{27-30} was visualized by chemiluminescence on sensitive films. PrP^{27-30} traces in the S^{HS}, were recovered in the pellet after NaPTA precipitation (P^{NaPTA}). PK-treated 263K-infected Syrian hamster brain homogenate (B.H.) was loaded as positive control.

Infectivity measurement of 263K scrapie–infected brain fractions

Virtually all infectivity present in the S^{LS} fraction is recovered in the pellet after ultracentrifugation (P^{HS} fraction). A substantial amount of infectivity is also found in the S^{HS} fraction, however. The mean incubation periods of hamsters inoculated with aliquots of S^{HS} fractions were, respectively, 15.9, 18.5, and 25.6 days longer than the corresponding S^{LS} fractions (Table 2), which corresponds to an estimate lost of infectivity titer ranging from 30 to 200 times.

The enrichment factor for infectivity versus PrP^{TSE} (calculated as the difference between the reduction factor for PrP^{TSE} and the reduction factor for infectivity) ranged from more than 200 times in Replicate 2 up to 1000 times in Replicate 1 (Table 2). Virtually all infectivity in S^{HS} was recovered in the pellet (P^{NaPTA}) after NaPTA precipitation.

DISCUSSION

In blood of TSE-affected rodents or sheep, a substantial proportion of infectivity is associated with plasma.[20-22] If the distribution of infectivity in human blood is the same as in animals, then plasma-derived products might be at risk of transmitting vCJD. Usually, precautions against the risk of infection in medicinal products, including plasma-derived pharmaceuticals, consist of source deferrals, screening of donors, and inactivation or removal of the infectious agent. In TSE diseases, however, the first two lines of defense are poorly practicable because blood is infectious during the long asymptomatic phase of

TABLE 2. Bioassay measurement of infectivity in different fractions (S^{LS}, S^{HS}, and P^{HS}) of 263K scrapie–infected brain

Replicate number	Fraction	Number of days (± SD) in incubation periods (number)	Estimated titer (log LD_{50}/mL of 10% brain suspension)	Difference (log) between S^{LS} and S^{HS}	Enrichment factor (infectivity/PrP^{27-30})
1	S^{LS}	55.8 ± 1.0 (8)	8.4	1.5	$\geq 10^{3.0}$
	S^{HS}	71.7 ± 2.3 (9)	6.9		
	P^{HS}	56.4 ± 2.7 (10)	8.3		
2*	S^{LS}	56.5 ± 2.0 (10)	8.3	1.7	$\geq 10^{2.3}$
	S^{HS}	75.0 ± 0.0 (9)	6.6		
	P^{HS}	59.1 ± 6.9 (10)	8.0		
3	S^{LS}	62.3 ± 0.7 (7)	7.7	2.3	$\geq 10^{2.7}$
	S^{HS}	87.9 ± 1.9 (7)	5.4		
	P^{HS}	Not done			
4†	S^{HS}	85.6 ± 4.2 (7)	6.6		
	P^{NaPTA}	87.7 ± 4.6 (7)	6.4		

* No sonication was performed in this replicate.
† Samples were 10-fold diluted respect to other replicates.

disease[1,2] and no tests are yet available for an early preclinical diagnosis[3,8] or for the screening of blood.[10,11] Thus, efforts are directed to implement procedures able to remove or inactivate TSE agents. Validation studies performed in the past years suggest that TSE agents can be removed by the processes used to manufacture plasma products. There is uncertainty, however, on the complete validity of these experiments mainly because it has been questioned whether the TSE agents in exogenous infectious materials used to spike human blood share the same physicochemical characteristics of the vCJD and other TSE agents in blood.[12,23] A comparison of different spiking preparations showed that brain homogenate, caveolae-like domains, and microsomes partition similarly, whereas purified PrP^{TSE} had significantly different partitioning properties.[23] Obviously, the best spiking material would be infectious human plasma,[24] but all attempts to transmit the disease with whole blood or buffy coat from human patients to experimental animals have so far failed.[25,26] The next best is to use blood from TSE-infected rodents. There is long-lasting evidence that blood of hamsters with experimental scrapie,[14,27] mice with experimental Gerstmann-Sträussler-Scheinker disease[20,28] or vCJD,[13] and sheep with natural scrapie[21] or experimental BSE[21,29] is infectious. Their blood contains too little infectivity to ensure the efficacy of removal procedures, making mandatory the use of exogenous spiking materials to perform reliable validation studies. Then, considering that removal may be influenced by the state of prion aggregation,[30] what is the most appropriate spiking material for the validation of the processes used for manufacturing plasma products? Brain homogenate may not be relevant because it contains large cell and membrane debris, high lipid content, and other brain molecules. Neither are highly purified PrP^{TSE} aggregates since they are not likely to be present in blood. Any attempt to measure PrP^{TSE} in blood or concentrates of blood components, such as buffy coats, has been frustrating, and claims of success have not been successively confirmed.[10] PrP^{TSE} in buffy coat of diseased 263K scrapie–infected hamsters is detectable after at least 144 cycles of protein misfolding cyclic amplification.[31] Theoretically, microsomal membrane fraction is a better spiking material,[12] although data from rodents infected with a mouse-adapted strain of human Gerstmann-Sträussler-Scheinker disease have shown that plasma is free of membranous structures,[32] that filtration or high-speed centrifugation does not eliminate infectivity from plasma,[42] and that in plasma of vCJD-infected mice, infectivity is reduced by PK treatment.[33] These data suggest that in plasma the infectious agent is very small, unsedimentable, and poorly aggregated.

In this scenario, the S^{IIS} fraction purified from 263K scrapie–infected hamster brains may be an appropriate spiking material for these studies. S^{IIS} fraction contains at least 10,000-fold the infectivity found in blood of TSE-infected rodents; it is virtually free from PrP^{TSE} aggregates, membranous fractions, and detergent contaminants, which interferes with the efficacy of TSE removal during the production of plasma derivatives.[12,34,35] The finding that in S^{IIS} fraction TSE infectivity is dissociated from PrP^{TSE} aggregates is not surprising, although the primary consequence of an infection with a TSE agent is the conformational change of the cellular PrP into a pathological conformer (PrP^{TSE}), rich in β-sheet structures, which tends to aggregate into amyloid fibers[36] and cosegregates with infectivity.[37] Exceptions to this rule, i.e., the presence of infectivity without the formation of PrP^{TSE} aggregates, have been reported,[38–42] and blood might simply be another condition where this divergence occurs. Likely, the infectivity in S^{IIS} is associated with dimer or small aggregates of PrP^{TSE} that remain in solution after ultracentrifuge, but precipitate in the presence of NaPTA and Mg^{2+}, which form complexes with PrP^{TSE} but not with cellular prion protein.[43]

It is therefore likely that an efficient removal of infectivity through nanofilters or depth filtrations[44–46] is achieved only when infectivity is associated to PrP^{TSE} in its aggregate form, but that these procedures may not be so effective when applied to naturally infected blood or plasma units. In conclusion, this study shows a simple and fast method for preparing a suitable spiking material to use in validation experiments aimed at proving removal or inactivation of prion infectivity in the preparation of blood components or pharmaceuticals derived from human plasma.

ACKNOWLEDGMENT

We are grateful to Fabiana Superti PhD and Antonella Tinari for electron microscopy; Nicola Bellizzi and Maurizio Bonanno for assistance in the bioassay; Ferdinando Costa, Patrizia Cocco, and Daniela Diamanti for laboratory work; and Alessandra Garozzo and Marco Del Re for administrative support.

REFERENCES

1. Llewelyn CA, Hewitt PE, Knight RS, et al. Possible transmission of variant Creutzfeldt-Jakob disease by blood transfusion. Lancet 2004;363:417-21.
2. Peden AH, Head MW, Ritchie DL, Bell JE, Ironside JW. Preclinical vCJD after blood transfusion in a PRNP codon 129 heterozygous patient. Lancet 2004;364:527-9.
3. Collinge J. Molecular neurology of prion disease. J Neurol Neurosurg Psychiatry 2005;76:906-19.
4. The National Creutzfeldt-Jakob Disease Surveillance Unit [homepage on the Internet]. Edinburgh: The University of Edinburgh; c2005 [updated 2005 Dec 6]. Available from: http://www.cjd.ed.ac.uk/
5. The European and Allied Countries Collaborative Study Group of CJD (EUROCJD) [homepage on the Internet].

Edinburgh: National CJD Surveillance Unit; c2005 [updated 2005 Nov 2]. Available from: http://www.eurocjd.ed.ac.uk/EUROINDEX.htm
6. La Bella V, Collinge J, Pocchiari M, Piccoli F. Variant Creutzfeldt-Jakob disease in an Italian woman. Lancet 2002;360:997-8.
7. Chadeau-Hyam M, Alperovitch A. Risk of variant Creutzfeldt-Jakob disease in France. Int J Epidemiol 2005;34:46-52.
8. Knight RSG, Will RG. Prion diseases. J Neurol Neurosurg Psychiatry 2004;75(Suppl 1):i36-42.
9. Hilton DA, Ghani AC, Conyers L, et al. Prevalence of lymphoreticular prion protein accumulation in UK tissue samples. J Pathol 2004;203:733-9.
10. Brown P, Cervenakova L. The modern landscape of transfusion-related iatrogenic Creutzfeldt-Jakob disease and blood screening tests. Curr Opin Hematol 2004;11: 351-6.
11. MacGregor IR. Screening assays for transmissible spongiform encephalopathies (TSEs). Vox Sang 2004;87(Suppl 2):3-6.
12. Foster PR. Removal of TSE agents from blood products. Vox Sang 2004;87(Suppl 2):7-10.
13. Cervenakova L, Yakovleva O, McKenzie C, et al. Similar levels of infectivity in the blood of mice infected with human-derived vCJD and GSS strains of transmissible spongiform encephalopathy. Transfusion 2003;43:1687-94.
14. Casaccia P, Ladogana A, Xi YG, Pocchiari M. Levels of infectivity in the blood throughout the incubation period of hamsters peripherally injected with scrapie. Arch Virol 1989;108:145-9.
15. Lee DC, Stenland CJ, Hartwell RC, et al. Monitoring plasma processing steps with a sensitive Western blot assay for the detection of the prion protein. J Virol Methods 2000;84:77-89.
16. Kascsak RJ, Rubenstein R, Merz PA, et al. Mouse polyclonal and monoclonal antibody to scrapie-associated fibril proteins. J Virol 1987;61:3688-93.
17. Wadsworth JD, Joiner S, Hill AF, et al. Tissue distribution of protease resistant prion protein in variant Creutzfeldt-Jakob disease using a highly sensitive immunoblotting assay. Lancet 2001;358:171-80.
18. Pocchiari M, Schmittinger S, Masullo C. Amphotericin B delays the incubation period of scrapie in intracerebrally inoculated hamsters. J Gen Virol 1987;68:219-23.
19. Pocchiari M, Casaccia P, Ladogana A. Amphotericin B: a novel class of antiscrapie drugs. J Infect Dis 1989;160:795-802.
20. Brown P, Rohwer RG, Dunstan BC, et al. The distribution of infectivity in blood components and plasma derivatives in experimental models of transmissible spongiform encephalopathy. Transfusion 1998;38:810-6.
21. Hunter N, Foster J, Chong A, et al. Transmission of prion diseases by blood transfusion. J Gen Virol 2002;83:2897-905.
22. Gregori L, McCombie N, Palmer D, et al. Effectiveness of leucoreduction for removal of infectivity of transmissible spongiform encephalopathies from blood. Lancet 2004;364: 529-31.
23. Vey M, Baron H, Weimer T, Groner A. Purity of spiking agent affects partitioning of prions in plasma protein purification. Biologicals 2002;30:187-96.
24. Lee DC, Stenland CJ, Miller JLC, et al. A direct relationship between the partitioning of the pathogenic prion protein and transmissible spongiform encephalopathy infectivity during the purification of plasma proteins [published erratum appears in Transfusion 2001;41:1079]. Transfusion 2001;41:449-55.
25. Bruce ME, McConnell I, Will RG, Ironside JW. Detection of variant Creutzfeldt-Jakob disease infectivity in extraneural tissues. Lancet 2001;358:208-9.
26. Brown P, Cervenakova L, Diringer H. Blood infectivity and the prospects for a diagnostic screening test in Creutzfeldt-Jakob disease [published erratum appears in J Lab Clin Med 2001;137:230]. J Lab Clin Med 2001;137:5-13.
27. Diringer H. Sustained viremia in experimental hamster scrapie. Arch Virol 1984;82:105-9.
28. Kuroda Y, Gibbs CJ Jr, Amyx HL, Gajdusek DC. Creutzfeldt-Jakob disease in mice: persistent viremia and preferential replication of virus in low-density lymphocytes. Infect Immun 1983;41:154-61.
29. Houston F, Foster JD, Chong A, Hunter N, Bostock CJ. Transmission of BSE by blood transfusion in sheep. Lancet 2000;356:999-1000.
30. Burnouf T, Radosevich M. Nanofiltration of plasma-derived biopharmaceutical products. Haemophilia 2003;9:24-37.
31. Castilla J, Saá P, Soto C. Detection of prions in blood. Nat Med 2005;11:982-5.
32. Brown P, Cervenakova L, McShane LM, et al. Further studies of blood infectivity in an experimental model of transmissible spongiform encephalopathy, with an explanation of why blood components do not transmit Creutzfeldt-Jakob disease in humans. Transfusion 1999;39:1169-78.
33. Yakovleva O, Janiak A, McKenzie C, et al. Effect of protease treatment on plasma infectivity in variant Creutzfeldt-Jakob disease mice. Transfusion 2004;44:1700-5.
34. Tateishi J, Kitamoto T, Mohri S, et al. Scrapie removal using Planova virus removal filters. Biologicals 2001;29: 17-25.
35. Van Holten RW, Autenrieth S, Boose JA, Hsieh WT, Dolan S. Removal of prion challenge from an immune globulin preparation by use of a size-exclusion filter. Transfusion 2002;42:999-1004.
36. Pan KM, Baldwin M, Nguyen J, et al. Conversion of α-helices into β-sheets features in the formation of the scrapie prion proteins. Proc Natl Acad Sci U S A 1993;90:10962-6.
37. McKinley MP, Bolton DC, Prusiner SB. A protease-resistant protein is a structural component of the scrapie prion. Cell 1983;35:57-62.
38. Manuelidis L, Sklaviadis T, Manuelidis EE. Evidence

suggesting that PrP is not the infectious agent in Creutzfeldt-Jakob disease. EMBO J 1987;6:341-7.
39. Xi YG, Ingrosso L, Ladogana A, Masullo C, Pocchiari M. Amphotericin B treatment dissociates in vivo replication of the scrapie agent from PrP accumulation. Nature 1992;356:598-601.
40. Wille H, Zhang GF, Baldwin MA, Cohen FE, Prusiner SB. Separation of scrapie prion infectivity from PrP amyloid polymers. J Mol Biol 1996;259:608-21.
41. Lasmezas CI, Deslys JP, Robain O, et al. Transmission of the BSE agent to mice in the absence of detectable abnormal prion protein. Science 1997;275:402-5.
42. Shaked GM, Fridlander G, Meiner Z, Taraboulos A, Gabizon R. Protease-resistant and detergent-insoluble prion protein is not necessarily associated with prion infectivity. J Biol Chem 1999;274:17981-6.
43. Safar J, Wille H, Itri V, et al. Eight prion strains have PrP^{Sc} molecules with different conformations. Nat Med 1998;4: 1157-65.
44. Foster PR, Griffin BD, Bienek C, et al. Distribution of a bovine spongiform encephalopathy-derived agent over ion-exchange chromatography used in the preparation of concentrates of fibrinogen and factor VIII. Vox Sang 2004;86:92-9.
45. Stenland CJ, Lee DC, Brown P, Petteway SR Jr, Rubenstein R. Partitioning of human and sheep forms of the pathogenic prion protein during the purification of therapeutic proteins from human plasma. Transfusion 2002;42:1497-500.
46. Wang K, Johnson A, Obradovic M, et al. TSE clearance during plasma products separation process by Gradiflow(TM). Biologicals 2005;33:87-94.

医薬品　研究報告 調査報告書

識別番号・報告回数			報告日	第一報入手日	新医薬品等の区分	総合機構処理欄
一般的名称	－		研究報告の公表状況	http://www.mhlw.go.jp/houdou/2006/08/h0824-3.html	公表国 日本	
販売名(企業名)	－					

研究報告の概要	使用上の注意記載状況・その他参考事項等
変異型クロイツフェルト・ヤコブ病(vCJD)は、献血の際に血液で検査する方法が未だ実用化されていないため、例えば、欧州滞在歴のある方などvCJD伝播のリスクが否定できない方について、問診により献血制限を行う暫定的な措置が講じられている。 今般、ヒト胎盤エキス(プラセンタ)注射剤を使用した方の取扱いについても、以下の措置を講じることとなった。 (1) 同注射剤によるvCJD感染事例は報告されていないが、輸血や臓器移植と同様にヒト由来の臓器から製造されていることから、vCJD伝播の理論的なリスクが否定できないため、念のための措置として、その使用者について問診により献血を制限する。 (2) 日本赤十字社においては、1カ月後を目途に措置を実施する予定。	重要な基本的注意 現在までに本剤の投与により変異型クロイツフェルト・ヤコブ病（vCJD）等が伝播したとの報告はない。しかしながら、製造工程において異常プリオンを低減し得るとの報告があるものの、理論的なvCJD等の伝播のリスクを完全には排除できないので、投与の際には患者への説明を十分行い、治療上の必要性を十分検討の上投与すること。

報告企業の意見	今後の対応
ヒト胎盤エキス(プラセンタ)注射剤 からのvCJD伝播の理論的なリスクが否定できないため、念のための措置の情報である。 現時点まで血漿分画製剤からのvCJD伝播の報告はない。	今後ともvCJDに関する安全性情報、規制情報等に留意していく。

報道発表資料

平成18年8月24日
（連絡先）
医薬食品局血液対策課
課長　関　英一
（内）　2900
（直通）03-3595-2395

ヒト胎盤エキス（プラセンタ）注射剤使用者の献血制限について

平成18年8月23日に開催された薬事・食品衛生審議会血液事業部会安全技術調査会において、ヒト胎盤エキス（プラセンタ）注射剤を使用した方の献血を制限する措置を日本赤十字社が実施することが了承された。

1．経緯等

(1) 変異型クロイツフェルトヤコブ病（vCJD）は、献血の際に血液から検査する方法が未だ実用化していないため、例えば、欧州滞在歴のある方などvCJD伝播のリスクが否定できない方について、問診により献血制限を行う暫定的な措置を講じてきているところである。

（※）暫定的な措置の内容
- 平成17年2月に国内でvCJD患者が確認され、英国滞在歴を有していたことを踏まえ、同年6月より、特定の期間に1日以上英国滞在歴のある方の献血を制限。
- 輸血及び臓器移植（ヒトの臓器に由来するもの）を受けた方からの献血を制限。

(2) ヒト胎盤エキス（プラセンタ）注射剤を使用した方の取扱いについても、安全技術調査会において平成16年10月から審議されてきたところであり、今般、以下の措置を講じることとしたものである。

2．新たな措置の内容

(1) 同注射剤によるvCJD感染事例は報告されていないが、輸血や臓器移植と同様にヒト由来の臓器から製造されていることから、vCJDの伝播の理論的なリスクが否定できないため、念のための措置として、その使用者について問診により献血を制限することとする。

（注）ヒト胎盤エキス（プラセンタ）注射剤については、国内では2製剤が薬事法の承認を受けている。
[1] メルスモン（注射薬）（メルスモン）
効能・効果　更年期障害・乳汁分泌不全
[2] ラエンネック（注射薬）（日本生物製剤）
効能・効果　慢性肝疾患における肝機能の改善
※　美容形成（シミ・シワ・ニキビ等）に一部使われていることも知られている。

(2) 日本赤十字社においては、1ヶ月後を目途に措置を実施する予定である。

→ 関連資料 … 薬事・食品衛生審議会　平成18年度第1回血液事業部会安全技術調査会

トップへ

報道発表資料　厚生労働省ホームページ

医薬品
医薬部外品　研究報告　調査報告書
化粧品

別紙 3-1

識別番号・報告回数		回	報告日 年　月　日	第一報入手日 2006 年 2 月 28 日	新医薬品等の区分 該当なし	総合機構処理欄
一般的名称			研究報告の公表状況	Emerging viral diseases and infectious disease risks Tapper, M.L. Haemophilia 12, (Suppl. 1), 3 - 7 (2006)	公表国 米国	
販売名（企業名）						

研究報告の概要	使用上の注意記載状況・その他参考事項等
西ナイルウイルス感染症，鳥インフルエンザ，重症急性呼吸器症候群(SARS)に特に重点をおいた「新規感染症」の概念，及びそれに関連すると思われる血液製剤とその使用患者の安全性について取り上げている。1992年以来，米国の医学研究所(IOM)は「新規感染症は，新型の，または再興する，または薬剤耐性の感染症であり，ヒトへの罹患率が過去20年以内に増加しているか，近い将来増加する恐れがある疾患」と定義している。海外旅行や国際商取引，人口統計学上及びそれに付随した行動の劇的な変化により感染因子は世界的に蔓延し，加速している。 2002年以来，輸血に関連する西ナイルウイルス感染症報告を受けて，米国においてウイルス検査を行った結果，多くの感染供血者(献血時には無症候であった)が特定された。その一方，SARSウイルスとトリインフルエンザウイルスでは，現時点で安全な血液供給に影響を及ぼす事態は生じていない。しかし，血液供給と血液由来製剤の安全性を脅かす新興病原体の検出と除去に対して厳重に警戒するよう提言している。	BYL-2006-0220-1

報告企業の意見	今後の対応
血漿分画製剤を介した西ナイルウイルス感染は現在までにまったく報告されていない。また，2003 年 5 月 1 日の FDA ガイドラインでは，血漿分画製剤に使用する血漿プールに対しては西ナイルウイルスの検査は必要ないとしている。さらに，弊社のウイルス不活化処理は血漿プールにおいて，西ナイルウイルスを不活化させるのに十分であることが証明されている。インフルエンザウイルス及びコロナウイルスに関しても同様に，弊社のウイルス不活化処理またはウイルス除去処理により安全性が確保されている。	現時点で新たな安全対策上の措置を講じる必要は無いと考える。引き続き関連情報の収集に努める。

Haemophilia (2006), 12, (Suppl. 1), 3–7

Emerging viral diseases and infectious disease risks

M. L. TAPPER
Division of Infectious Diseases and Hospital, Epidemiology, Lenox Hill Hospital, New York, NY, USA

Summary. New pathogens and antimicrobial-resistant forms of older pathogens continue to emerge, some with the potential for rapid, global spread and high morbidity and mortality. Pathogens can emerge either through introduction into a new population or when the interaction with the vector changes; emergence is also influenced by microbiological adaptation and change, global travel patterns, domestic and wild animal contact and other variants in human ecology and behaviour. Quick, decisive action to detect and control novel pathogens, and thereby contain outbreaks and prevent further transmission, is frequently hampered by incomplete or inadequate data about a new or re-emerging pathogen. Three examples of pathogens that are current causes for human health concern are avian influenza, West Nile virus (WNV) and the severe acute respiratory syndrome (SARS) coronavirus. Pathogens directly or indirectly transmitted by aerosolized droplets, such as avian influenza and SARS, pose considerable containment challenges. Rapid screening tests for other newly described pathogens such as WNV require time for development and may be <100% reliable. The importance of vigilance in the detection and control of newly recognized infectious threats cannot be overstressed. The presence of infectious agents in the blood supply could again have a significant impact on the safe use of both blood and blood-derived products in the care of patients with haemophilia, as did the human immunodeficiency virus in the 1980s. Emerging pathogens will continue to be a reality requiring the collaborative efforts of public health and individual healthcare providers worldwide to contain outbreaks and prevent transmission.

Keywords: avian influenza, haemophilia, human immunodeficiency virus, pathogens, severe acute respiratory syndrome, West Nile virus

Introduction

The emergence of new infectious pathogens and the recurrence of older pathogens in unique settings have become common topics in the medical literature and lay media, indicating an increasing concern among healthcare providers and the general public alike. The presence of infectious agents in the blood supply, for example, has had – and could again have – a profound influence on the safe use of both blood and blood-derived products in the care of patients with haemophilia. This article provides an overview of emerging infectious diseases in general and discusses some examples of viral pathogens that are currently cause for concern, including West Nile virus (WNV), severe acute respiratory syndrome (SARS) and avian influenza. It also lays the foundation for discussions about the implications of emerging infectious diseases for the safety of the blood supply and for the care of patients who depend on the safety of the blood supply, such as those with haemophilia.

Correspondence: Michael L. Tapper, MD, Division of Infectious Diseases and Hospital, Epidemiology, Lenox Hill Hospital, 100 East 77th Street, New York, NY 10021, USA.
Tel.: +1 212 434 3440; fax: +1 212 434 2674
e-mail: mtapper@lenoxhill.net

Infectious disease outbreaks of the last decade

In the last decade there have been a number of major global infectious disease outbreaks that have had the potential to be major health threats. Many of these rapidly spreading viruses, including SARS and avian influenza, appear to have originated as zoonoses in Asia [1]. These viruses have also demonstrated an extraordinary capacity to move quickly (and often surreptitiously) between animal and human populations and across continents.

Definition of an emerging infectious disease

Defining an emerging infectious disease is not necessarily straightforward. Morbidity and mortality from

emerging infectious diseases are understood to be a continual threat, yet the exact nature of that threat is not well defined. One widely accepted definition was proposed in 1992 by the Institute of Medicine (IOM) in the USA, which defined an emerging infectious disease as a new, re-emerging, or drug-resistant infection whose incidence in humans has increased within the past two decades or whose incidence has threatened to increase within the near future [2]. Based on this definition, a spectrum of potential infectious diseases becomes apparent.

Potential infectious disease threats

A continuum exists in types of pathogens that emerge and infect new populations. The continuum includes infectious diseases such as SARS that appear to be newly introduced to humans from animals as well as bioengineered organisms that produce disease in unforeseen ways, such as the transmission of anthrax by contaminated mail in the USA in 2001. Outbreaks of disease once thought to be well controlled may be associated with a breakdown in core public health measures such as treatment of established infection (e.g. tuberculosis) or routine childhood immunizations (poliomyelitis) The continuum of potential disease threats also includes new antimicrobial-resistant forms of established pathogens, such as methicillin-resistant *Staphylococcus aureus*. In addition, scientists continue to recognize previously unidentified infectious origins of some chronic diseases, such as Lyme borreliosis [3].

Factors contributing to emerging infections

In 1992 the IOM identified numerous factors that contribute to emerging infectious diseases, all of which may impact the safety of the blood supply [2]. These factors include:

1 human demographics and behaviour;
2 technology and industry;
3 economic development and land use;
4 international travel and commerce;
5 microbiological adaptation and change;
6 breakdown of core public health measures.

In 2003, the IOM published an update to the 1992 report in which additional contributing factors were identified [3]:

1 human susceptibility to infection;
2 climate and weather;
3 changing ecosystems;
4 poverty and social inequality;
5 war and famine;
6 lack of political will;
7 intent to harm.

Many of these factors are interdependent. International travel and commerce and human demographics and behaviour, for example, are closely related and have undergone considerable change in the last century. Over the last 150 years as the global population has increased dramatically, the length of time required to circumnavigate the globe has decreased dramatically (Fig. 1) [4]. International travel and commerce have affected the size and mobility of human populations, bringing some environments, humans and other animal species into contact with each other for the first time. These changing human demographics may enable an infectious agent to become adapted to and disseminated within a new host population, often resulting in an expansion of the agent's geographic range [5]. The combination of these factors has accelerated the global spread of infectious agents.

Route of transmission of emerging infectious disease

Emergence of an infectious disease can occur either through its introduction into a new population or when the interaction with the vector of a disease changes. The latter scenario is the likely manner in which viruses such as WNV and Lyme borreliosis have spread [5]. The WNV strain found in the USA, for example, is believed to have spread from the Middle East and be a variant of the virus first isolated in 1937 in the West Nile District of Uganda in Africa. It is uncertain how WNV spread to the USA. It has been hypothesized that the strain in the USA was

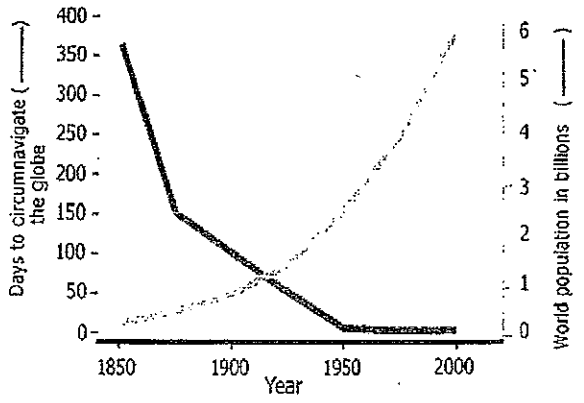


Fig. 1. Speed of global travel in relation to world population growth [4].

transported in an infected bird illegally imported from the Middle East or Central Europe where the disease had previously been endemic. Mosquito transmission subsequently resulted in transmission to birds, horses and humans in the USA. After its initial appearance in New York City in 1999, WNV spread to the lower 48 states in the US in <2 years [6].

Recent infectious disease concerns

New, emerging infectious diseases and disease agents continue to be discovered and described. While incomplete, the list in Table 1 provides an indication of the variety and quantity of pathogens that confront public health officials and present potential threats to human health [3].

West Nile virus

In 1999, the first cases of WNV infection were recognized in New York City. Over the next several years, the virus spread throughout the northeastern part of the country and subsequently spread west to the Mississippi River and south into Florida. By 2002, cases were being reported across most of the Midwest, and by 2005 every state in the continental USA had reported cases of WNV in humans, birds, mammals or mosquitoes [7].

Since 2002, following reports of transfusion-associated WNV infections, the US blood supply has been screened for the virus. As of 15, November 2005, 382 presumptively viremic blood donors had been identified and reported to the US Centers for Disease Control and Prevention (CDC). These donors were generally asymptomatic for WNV infection at the time of blood donation but tested seropositive when pooled samples were screened using nucleic amplification technology (NAT). Some of these individuals subsequently developed clinical symptoms [8].

Table 1. Partial list of emerging infectious diseases and disease-causing agents*.

HIV/AIDS
Tuberculosis
Dengue
Malaria (resistant *Plasmodium falciparum*)
Severe acute respiratory syndrome
Cholera
Meningococcal meningitis
Cryptosporidiosis
Filoviruses (Ebola/Marburg)
Legionella pneumophila
Lyme disease
Poliomyelitis
Toxin producing streptococci and *Staphylococcus aureus*
Human Herpesvirus-8
Parvovirus B19
Hepatitis C
Arenaviruses (Lassa)
Cyclospora cayetanensis
Hantavirus (Sin Nombre)
New variant CJD (BSE)
Bunyaviruses (Rift Valley)
Rotavirus
Escherichia coli 0157:H7
Bartonella henselae (cat scratch disease)
Community acquired MRSA
Avian influenza (H5N1)
West Nile virus
Salmonella enteritidis

AIDS, acquired immunodeficiency syndrome; BSE, bovine spongiform encephalopathy; CJD, Creutzfeldt–Jakob disease; HIV, human immunodeficiency virus; MRSA, methicillin-resistant *Staphylococcus aureus*.
*Data adapted from Smolinski *et al.* [3].

Severe acute respiratory syndrome

At the outset of the SARS epidemic in Asia, a number of small mammals commonly maintained in open food markets in Canton were found to be infected with the SARS coronavirus. More recent data have suggested that certain species of bats native to China may be the definitive host of the virus in nature [9].

Severe acute respiratory syndrome was first recognized in Hanoi, Vietnam in February 2003, although it is now believed to have originated in the Guangdong Province in southeast China in November 2002 [10]. In late February 2003, the first case of SARS in Hong Kong was reported in a physician from the Guangdong Province, who travelled to Hong Kong for a wedding. While staying overnight in a local hotel, it appears he transmitted the virus to 12 people on his floor. Subsequent generations of infection from the physician (who died in a Hong Kong hospital 2 days after arriving at the hotel), his relatives and others staying in the hotel involved more than 95 healthcare workers and 100 close contacts in the city of Hong Kong [11].

The global spread was rapid. Other infected hotel guests subsequently travelled to Vietnam, where 37 healthcare workers and 21 close contacts became infected, and to Singapore, where 34 healthcare workers and 37 close contacts were infected [11]. Another returned to Canada, where a cluster of infections commenced in a local hospital, involving family members, healthcare workers and other patients. Ultimately, over 200 people in Canada were infected, approximately one-third of whom died [12].

Avian influenza

Avian influenza is a major potential threat to the populations of the world and may be the source of the next flu pandemic [13]. There were three major flu pandemics in the last century: the so-called 'Spanish flu' in 1918–1919, potentially responsible for up to 50 million deaths worldwide; the Asian flu in 1957–1958, responsible for approximately 70 000 deaths in the USA; and the Hong Kong flu in 1968–1969, responsible for 34 000 deaths nationwide. Many epidemiologists believe that the human population is overdue for a pandemic [14]. Figure 2 illustrates a timeline of the emergence of several strains of the influenza virus.

Since 1918 there have been a number of shifts in the influenza virus's haemagglutinin and neuraminidase components, its key antigens. Fifteen types of haemagglutinin (H1–H15) and nine types of neuraminidase (N1–N9) have been recognized. Combinations involving subtypes H1–H3 and N1–N2 have been responsible for both seasonal and epidemic outbreaks in humans. The definitive hosts of influenza in nature are non-domesticated birds, particularly ducks that carry H1–H15 type viruses. Direct bird-to-human (and to date, rare instances of human-to-human) transmission of avian influenza has been reported [15] with increasing frequency in the last two and a half years.

Mechanism of influenza antigenic shift

Influenza viruses undergo constant subtle evolution and mutation of their principal proteins, a process referred to as antigenic drift. In addition to this naturally occurring and random process, influenza strains from different host species can periodically recombine. Swine may serve as hosts for both human and duck influenza strains and hence can function as ideal mixing vessels for major antigenic recombination and the emergence of novel influenza strains. When such shifts or recombinations occur and result in a virus with the capacity to maintain ongoing transmission between humans, a major pandemic may occur [16].

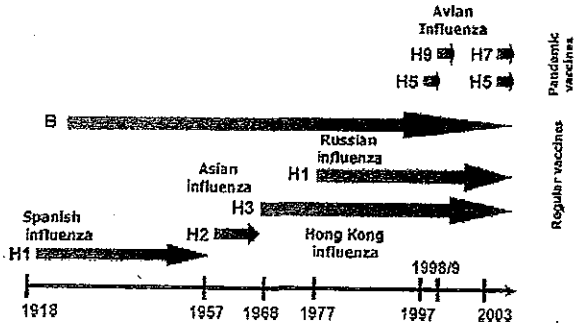


Fig. 2. Timeline of emergence of influenza viruses in humans. (Figure courtesy of the Centers for Disease Control and Prevention.)

In 1997 in Hong Kong, the first evidence emerged that avian viruses could directly infect humans without going through this interim mixing step [15,16]. In 1997, there was an outbreak of influenza associated with an avian (H5N1) strain in humans that was preceded by an outbreak of the same strain in poultry [17]. With six deaths among 18 hospitalizations, H5N1 exhibited unusual lethality and was considered by some public health officials and epidemiologists as a pandemic warning call.

By December 2003, confirmed cases of avian influenza among humans were reported in Vietnam and Thailand, and since January 2004, human cases have been reported in Vietnam, Thailand, Cambodia, Indonesia and the People's Republic of China. The total number of cases as of 17, November 2005 was 130, with 67 deaths [18]. Sustained outbreaks among domestic poultry flocks in Asia preceded these human cases.

While the major outbreaks of avian influenza have occurred among domestic poultry flocks, evidence of avian influenza viral infection in migrating birds throughout Asia (and more recently in Europe) has also been demonstrated. It has been suggested that migratory birds may be responsible for the widespread introduction of avian influenza into other bird populations, both domestic and wild [19].

Conclusion

New pathogens continue to emerge, some with the potential for rapid, global spread and high morbidity and mortality. Laboratory tests for viral detection can be developed once a virus is identified, but their development takes time and their reliability may be <100%.

Pathogens spread by aerosolized droplets, such as avian influenza and SARS, pose considerable containment challenges, although neither pathogen appears to clearly impact the safety of the blood supply. In the case of SARS, patients can be screened, but the exact mode of human-to-human transmission remains uncertain. In contrast, reasonably (although not universally) effective screening exists for some newly described blood-borne pathogens such as WNV. Nonetheless, the hard-learned lesson from the human immunodeficiency virus (HIV) experience in the 1980s is that the importance of vigilance in the

detection and elimination of newly recognized threats to blood safety cannot be overstressed. For these reasons, emerging pathogens will continue to be a reality requiring the best efforts of both public health officials and individual healthcare providers worldwide to identify emerging pathogens in a timely fashion, contain outbreaks and prevent transmission.

References

1 World Health Organization Global Influenza Program Surveillance Network. Evolution of H5N1 avian influenza viruses in Asia. *Emerg Infect Dis* (serial on the Internet) 2005; Published online at http://www.cdc.gov/ncidod/EID/vol11no10/05-0644.htm. Accessed September 2005.

2 Lederberg J, Shope RE, Oaks SC Jr, eds. *Emerging Infections: Microbial Threats to Health in the United States*. Washington, DC: National Academy Press, 1992; vii: 34.

3 Smolinski S, Hamburg MA, Lederberg J, eds. *Microbial Threats to Health: Emergence, Detection, and Response. Committee on Emerging Microbial Threats to Health in the 21st Century, Board on Global Health*. Washington, DC: National Academies Press, 2003: 54.

4 Murphy FA, Nathanson N. The emergence of new virus diseases: an overview. *Semin Virol* 1994; 5: 87–102.

5 Morse SS. Factors in the emergence of infectious diseases. *Emerg Infect Dis* 1995; 1: 7–15.

6 Tyler KL. West Nile virus encephalitis in America. Editorial. *N Engl J Med* 2001; 344: 1858–9.

7 Centers for Disease Control and Prevention. 2005 West Nile virus activity in the United States. Reported to CDC as of September 6, 2005. Published online at http://www.cdc.gov/ncidod/dvbid/westnile/surv&control05Maps.htm. Accessed October 2005.

8 Centers for Disease Control and Prevention, National Center for Infectious Diseases. 2005 West Nile virus viremic blood donor activity in the United States. Reported to the CDC as of November 15, 2005 Available at http://www.cdc.gov/ncidod/dvbid/westnile/surv&control05Maps_Viremic.htm. Accessed November 2005.

9 Bell D, Robertson S, Hunter P. Animal origins of SARS coronavirus: possible links with the international trade in small carnivores. *Philos Trans R Soc Lond B Biol Sci* 2004; 359: 1107–14.

10 World Health Organization. Severe acute respiratory syndrome (SARS) – multi-country outbreak – Update 24. April 8, 2003. Published online at http://www.who.int/csr/don/2003_04_08/en/. Accessed October 2005.

11 Centers for Disease Control and Prevention. Update: outbreak of severe acute respiratory syndrome – worldwide, 2003. *MMWR Morb Mortal Wkly Rep* 2003; 52: 241–8.

12 Varia M, Wilson M, Sarwal S *et al.* Investigation of a nosocomial outbreak of severe acute respiratory syndrome (SARS) in Toronto. *CMAJ* 2003; 169: 285–92.

13 Centers for Disease Control and Prevention. The influenza (flu) viruses. January 15, 2004. Published online at http://www.cdc.gov/flu/about/fluviruses.htm. Accessed September 2005.

14 Centers for Disease Control and Prevention. Key facts about pandemic influenza. October 17, 2005. Published online at http://www.cdc.gov/flu/pandemic/keyfacts.htm. Accessed December 2005.

15 Centers for Disease Control and Prevention. Avian influenza infection in humans. October 17, 2005. Published online at http://www.cdc.gov/flu/avian/gen-info/avian-flu-humans.htm. Accessed October 2005.

16 World Health Organization. Avian influenza – fact sheet. January 15, 2004. Published online at http://www.who.int/csr/don/2004_01_15/en/. Accessed October 2005.

17 Wood JM, Major D, Newman RW *et al.* Preparation of vaccines against H5N1 influenza. *Vaccine* 2002; 20(Suppl. 2): S84–7.

18 World Health Organization. Cumulative number of confirmed human cases of avian influenza A/(H5N1) reported to WHO. October 10, 2005. Published online at http://www.who.int/csr/disease/avian_influenza/country/cases_table_2005_11_17/en/index.html. Accessed November 2005.

19 Food and Agriculture Organization of the United Nations. *Potential Risk of Highly Pathogenic Avian Influenza (HPAI) Spreading Through Wild Water Bird Migration*. Avian Influenza Bulletin no. 33. September 1, 2005. Published online at http://www.fao.org/ag/againfo/subjects/documents/ai/AVIbull033.pdf. Accessed September 2005.

番号：1

別紙様式第２－１

医薬品　研究報告　調査報告書

識別番号・報告回数		報告日	第一報入手日 平成 18 年 9 月 12 日	新医薬品等の区分 該当なし	機構処理欄
一般的名称	ﾃｸﾈﾁｳﾑ人血清ｱﾙﾌﾞﾐﾝ (^{99m}Tc)	研究報告の公表状況	WHO sites Media centre: Notes for the Media 5 September 2006	公表国 南アフリカ他	
販売名（企業名）	テクネアルブミンキット （第一 RI）				

研究報告の概要	WHO が病原性が強く、致死性の結核の全世界的な拡大防止の強化および措置を求めた。広範囲の薬剤耐性結核（XDR-TB）は世界の様々な地域で確認されており、とくに旧ソビエト連邦諸国やアジアにおいてもっとも多く確認されている。また南アフリカにおいて報告された XDR-TB 疑いとされた HIV 陽性患者群において極めて高い死亡率（53 例中 52 例死亡。ただし、HIV の合併や適切な医療行為の欠如や重症患者のみが確認されていることによる影響を受けている可能性あり。）が確認されている。

報告企業の意見	今後の対応
本報告は、既知で重大な感染症に関する報告であるが、高度に治療抵抗性であり、従来認められない非常に高い死亡率が確認されていることから、重大な感染症の発生傾向の変化を示す報告と考えられる。なお、結核は呼吸器疾患であり、くしゃみや咳により感染するものであり、血液を介して感染するものではないことから、ヒト血液を原料とする当該生物由来製品には直接関連しないと判断する。	本報告はヒト血液を原料とする血漿分画製剤とは直接関連するものではなく、現時点で特に自社の当該生物由来製品に関し、措置を行う必要はないと判断する。しかしながら、世界的に重要な問題として WHO 等でも取り上げていることもあるため、今後とも関連情報については、注目して、情報収集に努める。

使用上の注意記載状況・その他参考事項等
特になし 尚、後日（10/30）、追加情報として、南アフリカより報告された 53 例の患者は XDR-TB の現状の定義には合致していなかったとの報告を入手。 [WHO The Weekly Epidemiological Record, No41,2006,81,386-390 (13 October 2006)]

MedDRA Version(9.1)

Emergence of XDR-TB
WHO concern over extensive drug resistant TB strains that are virtually untreatable

5 SEPTEMBER 2006 | GENEVA -- The World Health Organization (WHO) has expressed concern over the emergence of virulent drug-resistant strains of tuberculosis (TB) and is calling for measures to be strengthened and implemented to prevent the global spread of the deadly TB strains. This follows research showing the extent of XDR-TB, a newly identified TB threat which leaves patients (including many people living with HIV) virtually untreatable using currently available anti-TB drugs.

Later this week, WHO will join other TB experts at a two-day meeting in South Africa (7-8 September) to assess the response required to critically address TB drug resistance, particularly in Africa, and will take part in a news conference scheduled for Thursday, 7 September in Johannesburg.

What is XDR-TB?

MDR-TB (Multidrug Resistant TB) describes strains of tuberculosis that are resistant to at least the two main first-line TB drugs - isoniazid and rifampicin. XDR-TB, or Extensive Drug Resistant TB (also referred to as Extreme Drug Resistance) is MDR-TB that is also resistant to three or more of the six classes of second-line drugs.

The description of XDR-TB was first used earlier in 2006, following a joint survey by WHO and the US Centers for Disease Control and Prevention (CDC).

Resistance to anti-TB drugs in populations is a phenomenon that occurs primarily due to poorly managed TB care. Problems include incorrect drug prescribing practices by providers, poor quality drugs or erratic supply of drugs, and also patient non-adherence.

What is the current evidence of XDR-TB?

Recent findings from a survey conducted by WHO and CDC on data from 2000-2004 found that XDR-TB has been identified in all regions of the world but is most frequent in the countries of the former Soviet Union and in Asia.

In the United States, 4% of MDR-TB cases met the criteria for XDR-TB.

In Latvia, a country with one of the highest rates of MDR-TB, 19% of MDR-TB cases met the XDR-TB criteria.

Separate data on a recent outbreak of XDR-TB in an HIV-positive population in Kwazulu-Natal in South Africa was characterized by alarmingly high mortality rates.

Of the 544 patients studied, 221 had MDR-TB. Of the 221 MDR-TB cases, 53 were defined as XDR-TB. Of the 53 patients, 44 had been tested for HIV and all were HIV-positive.

52 of 53 patients died, on average, within 25 days including those benefiting from antiretroviral drugs.

Scarce drug resistance data available from Africa indicate that while population prevalence of drug resistant TB appears to be low compared to Eastern Europe and Asia, drug resistance in the region is on the rise.

Given the underlying HIV epidemic, drug-resistant TB could have a severe impact on mortality in Africa and requires urgent preventative action.

What action is required to prevent XDR-TB?

XDR-TB poses a grave public health threat, especially in populations with high rates of HIV and where there are few health care resources. Recommendations outlined in the WHO Guidelines for the Programmatic Management of Drug Resistant Tuberculosis include:

- strengthen basic TB care to prevent the emergence of drug-resistance
- ensure prompt diagnosis and treatment of drug resistant cases to cure existing cases and prevent further transmission

- increase collaboration between HIV and TB control programmes to provide necessary prevention and care to co-infected patients
- increase investment in laboratory infrastructures to enable better detection and management of resistant cases.

The Expert Consultation on Drug Resistant TB, hosted by the South African Medical Research Council with support from WHO and CDC, takes place in Johannesburg, 7-8 September.

A news conference will be held at 12.30pm, Thursday, 7 September, at the conference venue: Sunnyside Park Hotel, Parktown, Johannesburg.

For more information contact:

Glenn Thomas
Stop TB Department
WHO
Mobile: +41 79 5 09 06 77
E-mail: thomasg@who.int

Dr Karin Weyer
Director, South African Medical Research Council
Unit for TB Operational and Policy Research
Tel: +27 12 3 39 85 50
Mobile: +27 82 4 60 88 36
E-mail: kweyer@mrc.ac.za

別紙様式第2

医薬品 研究報告 調査報告書

識別番号・報告回数		報告日	第一報入手日 2006年4月14日	新医薬品等の区分	厚生労働省処理欄
一般的名称	①乾燥濃縮人活性化プロテインC ②乾燥濃縮人血液凝固第IX因子	研究報告の公表状況	Pathogenicity of a highly pathogenic avian influenza virus, A/chicken/Yamaguchi/7/04 (H5N1) in different species of birds and mammals. Archives of Virology. 2006 Feb 26 [Epub ahead of print]	公表国 日本	
販売名(企業名)	①注射用アナクトC2,500単位 ②ノバクトM				

研究報告の概要

(問題点：高病原性トリインフルエンザウイルスH5N1は、マウスにも感染する。)

2004年初頭、H5N1ウイルスによる高病原性トリインフルエンザが日本の2つの農場とペットのニワトリの間で発生した。最初のアウトブレイク中に死んだニワトリから分離されたA/chicken/Yamaguchi/7/04及びその他のH5N1ウイルスをニワトリ、ウズラ、セキセイインコ、子ガモ、マウス、ミニブタに実験的に感染させてウイルスの病原性を評価した。結果、ウイルスはすべてのトリで高病原性を示した。マウスは感受性を示したが、死亡率は低かった。ミニブタには感染しなかった。

22匹のマウスに対して、H5N1ウイルスを経鼻で感染させた。22匹のうち、2匹が接種3日及び4日後に死亡した。死亡したマウスを死亡後に、また残りの生存した20匹のマウスを接種3日及び14日後に剖検し、ウイルスの組織分布を調べた。4日後に死亡したマウスは呼吸器官以外に腎臓と肝臓からウイルスが分離された。3日後に死亡したマウス及び3日後に剖検したマウスは呼吸器官でのみウイルスが分離され、14日後に剖検したマウスからはウイルスは分離されなかった。また、すべてのマウスにおいて、脳からのウイルスの分離はなかった。

報告企業の意見	今後の対応
別紙のとおり	今後とも情報収集に努め、本剤の安全性の確保を図っていきたい。

使用上の注意記載状況・その他参考事項等

記載なし

別紙

一般的名称	①乾燥濃縮人活性化プロテインC、②乾燥濃縮人血液凝固第IX因子
販売名（企業名）	①注射用アナクトC2,500単位、②ノバクトM
報告企業の意見	「注射用アナクトC2,500単位」及び「ノバクトM」は、有効成分である活性化プロテインCあるいは血液凝固第IX因子を精製するために、プロテインCあるいは血液凝固第IX因子に対するモノクローナル抗体をリガンドとするアフィニティクロマトグラフィを使用しており、それぞれのモノクローナル抗体の調製にマウスを用いている。これらのモノクローナル抗体を精製する際にウイルス除去膜ろ過を実施しており、一定のウイルス除去効果が期待される。 一方、当該製剤は、「血漿分画製剤のウイルスに対する安全性確保に関するガイドライン（医薬発第1047号、平成11年8月30日）」に従い、ウシウイルス性下痢ウイルス（BVDV）、仮性狂犬病ウイルス（PRV）、ブタパルボウイルス（PPV）、A型肝炎ウイルス（HAV）または脳心筋炎ウイルス（EMCV）をモデルウイルスとして、ウイルスプロセスバリデーションを実施した製造工程により製造されている。今回報告した高病原性トリインフルエンザH5N1は、エンベロープの有無、核酸の種類等からモデルウイルスとしてはBVDVが該当すると考えられるが、上記バリデーションの結果から、当該製剤の製造工程の内、上記のイムノアフィニティクロマトグラフィ工程の下流に導入されている加熱工程がウイルス不活化効果を有することを確認している。 また、これまでに当該製剤による高病原性トリインフルエンザH5N1感染の報告例は無い。 以上の点から、当該製剤は高病原性トリインフルエンザウイルスH5N1に対する安全性を確保していると考える。

Arch Virol (2006)
DOI 10.1007/s00705-005-0723-6

Archives of Virology
Printed in Austria

Pathogenicity of a highly pathogenic avian influenza virus, A/chicken/Yamaguchi/7/04 (H5N1) in different species of birds and mammals

N. Isoda[1], Y. Sakoda[1], N. Kishida[1], G.-R. Bai[1], K. Matsuda[2], T. Umemura[2], and H. Kida[1]

[1]Laboratory of Microbiology, Department of Disease Control, Graduate School of Veterinary Medicine, Hokkaido University, Sapporo, Japan
[2]Laboratory of Comparative Pathology, Graduate School of Veterinary Medicine, Hokkaido University, Sapporo, Japan

Received September 20, 2005; accepted December 26, 2005
Published online February 26, 2006

Summary. Outbreaks of highly pathogenic avian influenza (HPAI) have been occurring in domestic poultry in Asia since 1996. In the beginning of 2004, HPAI outbreaks were caused by H5N1 virus in two farms and a group of pet chickens in different areas of Japan. In the present study, the pathogenicity of A/chicken/Yamaguchi/7/04 (H5N1), which had been isolated from a dead chicken during the first outbreak in Japan, was assessed in chickens, quails, budgerigars, ducklings, mice, and miniature pigs by experimental infection. The virus was highly pathogenic to all the birds tested. Mice were susceptible to infection with a low mortality rate and miniature pigs were resistant to infection with the virus.

Introduction

A wide variety of species of birds and mammals are susceptible to influenza A virus infection. Viruses of all 16 hemagglutinin (HA) (H1–H16) and 9 neuraminidase (N1–N9) subtypes have been isolated from avian species [1, 6]. Aquatic birds are the natural reservoirs of influenza A viruses [12]. Influenza viruses are perpetuated in nature by continuing to circulate in migratory ducks and frozen lake water [9]. Based on the severity of the disease they cause in chickens, avian influenza viruses are divided into two groups, highly pathogenic and low pathogenic [1]. Low pathogenic avian influenza (LPAI) viruses replicate in limited tissues where host proteases such as trypsin-like enzymes are found. Highly pathogenic avian influenza (HPAI) viruses possess inserted multiple basic amino acid residues at the site of cleavage of their HAs into HA1 and HA2 by ubiquitous proteases such

as furin and PC6 [8, 25]. This cleavage confers infectivity to a greater number of tissues, leading to a severe systemic disease, characterized by high mortality [14]. The HPAI viruses are restricted to subtypes H5 and H7, and viruses of these two subtypes had been believed to be low pathogenic in the reservoir host, ducks, until HPAI H5N1 viruses were isolated from bar-headed geese, brown-headed gulls, and black-headed gulls, 2005, in China [4, 16].

Outbreaks of HPAI in poultry such as chickens and quails around the world have caused high mortality and substantial economic losses, thereby impacting negatively on the poultry industry [1, 27]. Outbreaks have occurred often in the last decade in North America, Europe, and Asia. In Asia, highly pathogenic H5N1 influenza viruses have been recognized since 1996 [28]. In 1997, HPAI viruses were directly transmitted from birds to humans in Hong Kong, signaling the necessity to clarify the ecology of avian influenza virus [26]. HPAI outbreaks again occurred during 2001–2002 in Hong Kong [24]. In 2004, HPAI outbreaks also occurred in Cambodia, China, Indonesia, Malaysia, Japan, Laos, South Korea, Thailand, and Vietnam [15]. The HPAI virus, A/chicken/Yamaguchi/7/04 (H5N1), isolated in Japan, 2004, was lethal to chickens [18]. The pathogenicity of this HPAI virus in birds other than chickens and in mammals is not known. In order to determine the pathogenicity of the virus in chickens, quails, budgerigars, ducklings, mice, and miniature pigs, and to compare the pathogenicity of this HPAI virus in those animals in parallel with that of other H5N1 influenza viruses, experimental infection was carried out in the present study.

Materials and methods

Viruses

Influenza virus strain A/chicken/Yamaguchi/7/04 (H5N1) (Ck/Yamaguchi/04) was isolated from a dead chicken during the first outbreak of HPAI in Japan and was provided by the National Institute of Animal Health (Ibaraki, Japan) [18]. A/duck/Yokohama/aq-10/03 (H5N1) (Dk/Yokohama/03), isolated from duck meat imported from China, was provided by the Animal Quarantine service (Kanagawa, Japan) [13, 19]. R(A/duck/Mongolia/54/01-A/duck/Mongolia/47/01) (H5N1) (R(Dk/Mong-Dk/Mong)) was a reassortant virus generated from A/duck/Mongolia/54/01 (H5N2) and A/duck/Mongolia/47/01 (H7N1) which were isolated in our laboratory from fecal samples of wild ducks in Mongolia [13]. These three viruses were propagated in 10-day-old embryonated chicken eggs for 48 h at 35 °C. The infectious allantoic fluid was used as inoculum for experimental infections of animals and for the preparation of purified virus.

Animals

Chickens (*Gallus gallus*), quails (*Coturnix japonica*), budgerigars (*Melopsittacus undulatus*), ducklings (*Ahas platyhnchos*), mice (*Mus musculus*), and miniature pigs (*Sus scrofa domestic*) were used for the experimental infection study. Specific pathogen-free white leghorn chickens were hatched and raised for four weeks in our laboratory. One-month-old quails and three-month-old budgerigars were purchased from pet shops. Three-day-old ducklings were purchased from a duck farm in Hokkaido, Japan. Six-week-old female BALB/c mice and two-month-old specific pathogen-free male miniature pigs were purchased from Japan SLC, Inc. (Shizuoka, Japan) and Nippon Institute for Biological Science (Yamanashi, Japan).

Animal experiments

Viruses were inoculated intranasally, at a 50% egg infectious dose (EID_{50}) of $10^{8.0}$, into birds and mammals. For the birds and miniature pigs, 0.1 ml of each H5N1 virus containing $10^{8.0}EID_{50}$ was inoculated intranasally. For the mice, 0.03 ml of each H5N1 virus containing $10^{8.0}EID_{50}$ was inoculated intranasally. As a negative control, phosphate buffered saline (PBS) was given to the birds and mammals as much volume as the virus suspension. Birds and mice were sacrificed at 3 and 14 days post-infection (p.i.). When animals were dead or sacrificed, trachea and lung (respiratory organs), liver, spleen, kidneys, colon, brain, heart, pancreas, and blood of each animal were collected aseptically and were used for the titration of virus and histopathological examination. For miniature pigs, nasal swabs were collected in minimal essential medium daily from day 1 p.i. to day 7 p.i., and were used for the titration of virus. Animals were housed in self-contained isolator units (Tokiwa Kagaku, Tokyo, Japan) at a BSL 3 biosafety facility at the Graduate School of Veterinary Medicine, Hokkaido University, Japan.

Virus titration

The tissue homogenates from birds and mice were inoculated into 10-day-old embryonated chicken eggs and incubated for 48 h at 35 °C. The titers of virus were calculated by the method of Reed and Muench [22] and expressed as the EID_{50} per gram of tissue. Viral titers of the nasal swab samples of the miniature pigs were calculated as the 50% tissue culture infectious dose ($TCID_{50}$) per ml for swab in MDCK cells.

Antibody detection

Serum samples treated with beta-propiolactone (Wako Pure Chemicals Industries, Ltd., Japan) at 37 °C for 3 h were examined for the presence of antibodies against H5 influenza virus by ELISA. The purified R(Dk/Mong-Dk/Mong) (H5N1) virus was used as antigen for ELISA according to Kida et al. [10]. ELISA titers were expressed as reciprocals of serum dilutions.

Histopathological examination

The tissues of birds and mammals were fixed in 20% formalin in PBS (pH 7.2), sectioned, and stained with hematoxylin and eosin for microscopic examination. For the detection of influenza virus antigens in the tissues, all the sections were stained using the streptavidin-biotin immunoperoxidase complex method (Histofine SAB-PO ®kit, Nichirei Corp., Tokyo) with rabbit anti-A/duck/Pennsylvania/10218/84 (H5N2) hyperimmune serum at a 1:1,000 dilution as the primary antibody.

Results

Chickens

All of the chickens inoculated with Ck/Yamaguchi/04 and Dk/Yokohama/03 died on day 2 and between day 2 p.i. and day 4 p.i. (2–4d), respectively, and virus was recovered from each of the tissues tested (respiratory organs, liver, kidneys, colon, and brain) (Table 1). Higher titers of viruses were detected in four of the five tissues of chickens inoculated with Ck/Yamaguchi/04 than in those with Dk/Yokohama/03. None of the chickens inoculated with R(Dk/Mong-Dk/Mong) had died by day 14 p.i., and virus was not recovered from any of the tissues at

Table 1. Virus recovery and antibody response from chickens inoculated with avian influenza virus

Inoculated virus	No. of animals	Days p.i.		Virus recovery[a]						Antibody response[b]
				Respiratory organs	Liver	Kidneys	Colon	Brain	Blood	
Ck/Yamaguchi/04 (H5N1)	6	2d	dead	6 (8.4)	6 (7.4)	6 (7.6)	6 (7.3)	6 (7.1)	ND[c]	ND
Dk/Yokohama/03	5	2–4d	dead	5 (7.1)	5 (5.8)	5 (6.4)	5 (5.8)	5 (7.7)	ND	ND
(H5N1)	1	3d	sacrificed	1 (6.8)	1 (6.5)	1 (7.2)	1 (7.2)	1 (8.0)	1 (7.3)	–
R(Dk/Mong-Dk/Mong)	3	3d	sacrificed	0	0	0	0	0	0	–
(H5N1)	3	14d	sacrificed	0	0	0	0	0	0	–

[a]Digit: number of animals in which each virus was isolated. Parenthesis: average of virus titers ($logEID_{50}/g$). 0 indicates no virus was isolated from animals

[b]Antibody detection was examined by ELISA. –: ELISA titer was below 40

[c]Not determined

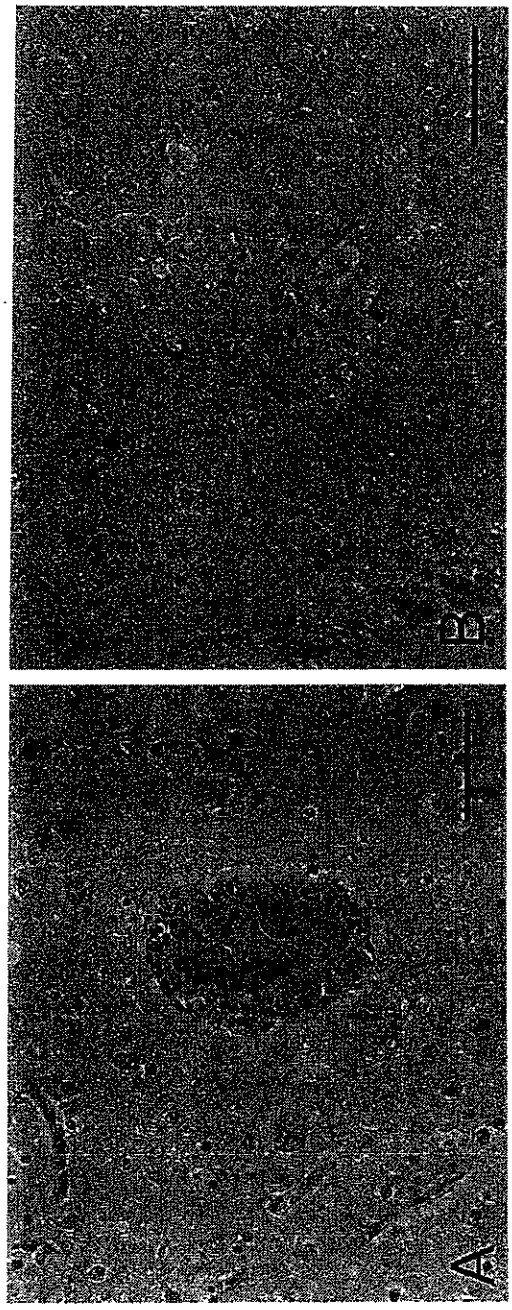


Fig. 1. Histopathological examination in chickens (A) and quails (B) inoculated with Dk/Yokohama/03. Photomicrographs of hematoxylin and eosin-stained tissue sections. A: Perivascular cuffing, swelling of endothelial cells, infiltration and proliferation of microglia in the brain (cerebrum) of the chickens inoculated with Dk/Yokohama/03 on day 4 p.i. B: Laminar encephalomalaccia (necrosis) in the brain (cerebellum) of the quails inoculated with Dk/Yokohama/03 on day 4 p.i. Bar, 50 μm

days 3 and 14 p.i. Sero-conversion to H5 influenza virus was not detected in any birds, indicating that the chickens were not infected with R(Dk/Mong-Dk/Mong). In the histopathological examination, influenza virus antigens were detected in the brain, liver, spleen, kidneys, heart, lungs, pancreas, and colon of chickens inoculated either with Ck/Yamaguchi/04 or with Dk/Yokohama/03. Since severe virus encephalitis with perivascular infiltration in the brain affected one chicken inoculated with Dk/Yokohama/03 (Fig. 1A) and higher titers were detected in the brains of chickens inoculated with Dk/Yokohama/03 than with Ck/Yamaguchi/04, it was found that infection with Dk/Yokohama/03 caused severer lesions than infection with Ck/Yamaguchi/04 in the brain.

Quails

All of the quails inoculated with Ck/Yamaguchi/04 and Dk/Yokohama/03 died between day 2 p.i. and day 3 p.i. (2–3d) and between day 3 p.i. and day 4 p.i. (3–4d), respectively, and virus was recovered from each of the tissues tested (Table 2). Disease signs characterized by severe nervous disorders were observed in 2 out of 6 quails infected with Dk/Yokohama/03. Higher titers of viruses were detected in all the tissues of quails inoculated with Ck/Yamaguchi/04 compared to those inoculated with Dk/Yokohama/03. None of the quails inoculated with R(Dk/Mong-Dk/Mong) had died by day 14 p.i., and virus was not recovered from any of the tissues at day 3 and 14 p.i. Sero-conversion to H5 influenza virus was detected in the quails inoculated with R(Dk/Mong-Dk/Mong) at day 14 p.i., indicating that these quails were infected with R(Dk/Mong-Dk/Mong). In the histopathological examination, in the brain of the quail inoculated with Dk/Yokohama/03, severe virus encephalitis with laminar encephalomalacia (necrosis) was observed (Fig. 1B). Antigens to influenza viruses were detected in the brains and hearts of birds infected either with Ck/Yamaguchi/04 or with Dk/Yokohama/03.

Budgerigars

All of the budgerigars inoculated either with Ck/Yamaguchi/04 or with Dk/Yokohama/03 died by day 5 p.i., and the virus was recovered from each of the tissues tested (Table 3). Disease signs such as severe nervous disorders were observed in 3 out of 7 budgerigars infected with Dk/Yokohama/03. None of the budgerigars inoculated with R(Dk/Mong-Dk/Mong) had died by day 14 p.i., and virus was not recovered from any of the tissues at days 3 and 14 p.i. Sero-conversion to H5 influenza virus was not detected in any budgerigars inoculated with R(Dk/Mong-Dk/Mong) at day 14 p.i., indicating that the budgerigars were not infected with R(Dk/Mong-Dk/Mong).

Ducklings

Two of the ducklings inoculated with Ck/Yamaguchi/04 died on day 6 p.i. and day 7 p.i. (6–7d), and virus was recovered from each of the tissues including

Table 2. Virus recovery and antibody response from quails inoculated with avian influenza virus

Inoculated virus	No. of animals	Days p.i.		Virus recovery[a]						Antibody response[b]
				Respiratory organs	Liver	Kidneys	Colon	Brain	Blood	
Ck/Yamaguchi/04 (H5N1)	7	2–3d	dead	7 (7.4)	7 (7.1)	7 (8.8)	7 (7.2)	7 (8.4)	ND[c]	ND
Dk/Yokohama/03	4	3–4d	dead	4 (6.8)	4 (4.4)	2 (5.7)	3 (6.4)	4 (8.3)	ND	ND
(H5N1)	2	3d	sacrificed	1 (7.2)	2 (6.0)	2 (8.0)	0	1 (5.8)	1 (3.8)	−
R(Dk/Mong-Dk/Mong)	3	3d	sacrificed	0	0	0	0	0	0	−
(H5N1)	2	14d	sacrificed	0	0	0	0	0	0	+

[a]Digit: number of animals in which each virus was isolated. Parenthesis: average of virus titers ($logEID_{50}/g$). 0 indicates no virus was isolated from animals

[b]Antibody detection was examined by ELISA. −: ELISA titer was below 40. +: ELISA titer was over 40

[c]Not determined

Table 3. Virus recovery and antibody response from budgerigars inoculated with avian influenza virus

Inoculated virus	No. of animals	Days p.i.		Virus recovery[a]					Antibody response[b]
				Respiratory organs	Liver	Kidneys	Colon	Brain	
Ck/Yamaguchi/04 (H5N1)	7	3–4d	dead	7 (6.6)	7 (4.3)	7 (7.1)	3 (3.8)	7 (7.4)	ND[c]
Dk/Yokohama/03 (H5N1)	4	3d	sacrificed	4 (5.0)	3 (3.5)	4 (5.4)	4 (2.9)	4 (6.2)	ND
	3	5d	dead	3 (5.3)	3 (2.6)	3 (4.9)	2 (2.9)	3 (8.0)	ND
R(Dk/Mong-Dk/Mong) (H5N1)	3	3d	sacrificed	0	0	0	0	0	ND
	3	14d	sacrificed	0	0	0	0	0	–

[a]Digit: number of animals in which each virus was isolated. Parenthesis: average of virus titers ($logEID_{50}/g$). 0 indicates no virus was isolated from animals

[b]Antibody detection was examined by ELISA. –: ELISA titer was below 40

[c]Not determined

Table 4. Virus recovery and antibody response from ducklings inoculated with avian influenza virus

Inoculated virus	No. of animals	Days p.i.		Virus recovery[a]					Antibody response[b]
				Respiratory organs	Liver	Kidneys	Colon	Brain	
Ck/Yamaguchi/04 (H5N1)	3	3d	sacrificed	3 (5.8)	3 (5.3)	3 (5.2)	3 (3.0)	0	–
	2	6–7d	dead	2 (3.9)	1 (5.5)	1 (5.7)	1 (2.5)	1 (5.3)	ND[c]
	1	14d	sacrificed	0	0	0	0	0	+
Dk/Yokohama/03 (H5N1)	6	3–4d	dead	6 (7.1)	6 (7.1)	6 (5.7)	6 (4.7)	6 (8.1)	ND
R(Dk/Mong-Dk/Mong) (H5N1)	3	3d	sacrificed	0	0	0	0	0	–
	3	14d	sacrificed	0	0	0	0	0	+

[a]Digit: number of animals in which each virus was isolated. Parenthesis: average of virus titers ($logEID_{50}/g$). 0 indicates no virus was isolated from animals

[b]Antibody detection was examined by ELISA. –: ELISA titer was below 40. +: ELISA titer was over 40

[c]Not determined

the brain (Table 4). One of the ducklings survived for 14 days, and from this duckling, specific serum antibodies against H5 influenza virus were detected. All of the ducklings inoculated with Dk/Yokohama/03 died between day 3 p.i. and day 4 p.i. (3–4d), and the virus was recovered from each tissue. None of the ducklings inoculated with R(Dk/Mong-Dk/Mong) had died by day 14 p.i., and virus was not recovered from any of the tissues at days 3 and 14 p.i. Seroconversion to H5 influenza virus was detected in the ducklings inoculated with R(Dk/Mong-Dk/Mong) at day 14 p.i.

Table 5. Virus recovery and antibody response from mice inoculated with avian influenza virus

Inoculated virus	No. of animals	Days p.i.		Virus recovery[a]					Antibody response[b]
				Respiratory organs	Liver	Spleen	Kidneys	Brain	
Ck/Yamaguchi/04	2	3–4d	dead	2 (6.3)	0	1 (3.3)	1 (2.3)	0	ND[c]
(H5N1)	2	3d	sacrificed	2 (6.7)	0	0	0	0	–
	4	14d	sacrificed	0	0	0	0	0	+
Dk/Yokohama/03	4	3d	sacrificed	3 (4.5)	0	0	0	0	–
(H5N1)	4	14d	sacrificed	0	0	0	0	0	+
R(Dk/Mong-	3	3d	sacrificed	3 (4.5)	0	0	0	0	ND
Dk/Mong) (H5N1)	3	14d	sacrificed	0	0	0	0	0	ND

[a]Digit: number of animals in which each virus was isolated. Parenthesis: average of virus titers ($logEID_{50}/g$). 0 indicates no virus was isolated from animals

[b]Antibody detection was examined by ELISA. –: ELISA titer was below 40. +: ELISA titer was over 40

[c]Not determined

Mice

Two of the mice inoculated with Ck/Yamaguchi/04 died on day 3 p.i. and day 4 p.i. (3–4d) (Table 5). Virus was recovered only from the respiratory organs in all except one mouse, which was dead at day 4 p.i. In this mouse, the virus was recovered not only from the respiratory organs but also from the spleen and kidneys. The other four mice survived for 14 days, and specific antibodies against H5 influenza virus were detected. All of the mice inoculated with Dk/Yokohama/03 and R(Dk/Mong-Dk/Mong) survived for 14 days. The virus was recovered only from the respiratory organs of the mice at day 3 p.i. It was found that the pathogenicity of these two viruses in mice was relatively low.

Table 6. Virus recovery and antibody response from miniature pigs inoculated with avian influenza virus

Viruses	Virus titers ($logTCID_{50}/ml$)							Antibody response[a] on 14 days p.i.
	1 day	2 day	3 day	4 day	5 day	6 day	7 day	
Ck/Yamaguchi/04 (H5N1)	–[b]	–	–	–	–	–	–	–
Dk/Yokohama/03 (H5N1)	–	–	–	–	–	–	–	–
R(Dk/Mong-Dk/Mong) (H5N1)	–	2.7	2.5	1.7	–	–	–	+

[a]Antibody detection was examined by ELISA. –: ELISA titer was below 40. +: ELISA titer was over 40

[b]–: <1.5 $logTCID_{50}/ml$

Miniature pigs

All of the miniature pigs inoculated with the three H5N1 viruses survived for 14 days. No virus was detected in the nasal swabs of the miniature pigs inoculated either with Ck/Yamaguchi/04 or Dk/Yokohama/03 from day 1 p.i. to day 7 p.i. (Table 6). In these two miniature pigs, sero-conversion to H5 influenza virus was not detected at day 14 p.i. In another experiment with miniature pigs inoculated with Ck/Yamaguchi/04, the virus was not recovered from any of the tissues at days 3 and 14 p.i. (data not shown). These results indicated that miniature pigs were not infected with Ck/Yamaguchi/04 and Dk/Yokohama/03. Although there were no disease signs in the miniature pig inoculated with R(Dk/Mong-Dk/Mong), viruses were recovered from the nasal swabs between day 2 p.i. and day 4 p.i. (2–4d). Sero-conversion to H5 influenza virus was detected in the miniature pig inoculated with R(Dk/Mong-Dk/Mong).

Discussion

The present study was conducted to determine the pathogenicity of Ck/Yamaguchi/04 in chickens, quails, budgerigars, ducklings, mice, and miniature pigs. Two H5N1 avian influenza viruses, Dk/Yokokama/03 and R(Dk/Mong-Dk/Mong), were compared in terms of pathogenicity with Ck/Yamaguchi/04. The intravenous pathogenicity index (IVPI) in 6-week-old chickens for Ck/Yamaguchi/04, Dk/Yokohama/03, and R(Dk/Mong-Dk/Mong) was 3.0, 2.7, and 0.0, respectively (data not shown). Based on the present results, Ck/Yamaguchi/04 and Dk/Yokohama/03 were classified as HPAI viruses and R(Dk/Mong-Dk/Mong) as a non-pathogenic virus by the OIE criteria [2]. Ck/Yamaguchi/04 and Dk/Yokohama/03 caused systemic infections in birds, but showed little or no pathogenicity in mammals. The slightly longer mean death time in chickens inoculated with Dk/Yokohama/03 allowed for the development of cyanosis of the wattle, typical signs of HPAI. The tendency was shown that virus of higher titer was recovered from chickens inoculated with Ck/Yamaguchi/04 than those inoculated with Dk/Yokohama/03.

The pathogenicity of Ck/Yamaguchi/04 and Dk/Yokohama/03 in quails and budgerigars was as high as that of the HPAI virus, Ck/Hong Kong/220/97 (H5N1), which caused an acute and lethal infection [21]. Notably, the pathogenicity of Ck/Yamaguchi/04 in the quails seemed to be higher than that of Dk/Yokohama/03, as evidenced by the mean death times (Ck/Yamaguchi/04 vs Dk/Yokohama/03, $P = 0.05$) and the tissues from which the viruses were recovered. This difference may be due to the adaptation of isolated HPAI viruses from different hosts (chicken and duck) to quails. The greater susceptibility of quails to the virus originating from duck than from chickens is consistent with previous reports [17]. In our another experiment, Ck/Yamaguchi/04 and Dk/Yokohama/03 caused systemic infections in wild starlings (*Sturnus cineraceus*) (data not shown), indicating that feral birds could play a role as intermediates in virus transmission among poultry flocks, thereby contributing to the spread of avian influenza virus as in the outbreaks in Australia [20]. During the outbreaks of H5N1 HPAI in Japan, 2004, viruses were

isolated not only from chickens but dead crows [18]. The possibility remains that avian influenza virus is spread by the contact of wild birds with chickens.

The pathogenicity of Ck/Yamaguchi/04 and Dk/Yokohama/03 for five-week-old ducks was not high compared to that for chickens and Dk/Yokohama/03 replicated more rapidly and efficiently in the multiple organs than Ck/Yamaguchi/04 in ducks [13]. In the present study, virus was recovered from multiple tissues of three-day-old ducklings inoculated either with Ck/Yamaguchi/04 or with Dk/Yokohama/03, and some of these ducklings were dead, indicating that the pathogenicity of these two viruses in three-day-old ducklings was high.

In the present study, virus was recovered from multiple tissues of only one mouse, which died at day 4 p.i. Two mice died after the inoculation of Ck/Yamaguchi/04 at an EID_{50} of $10^{8.0}$ and the mortality rate of mice was only 33% ($n=6$). In the latest publication, the 50% lethal dose of the same strain in mice was 5×10^5 EID_{50} under the same conditions (6-week-old female BALB/c mice via the intranasal route), and virus was also recovered from the brain [18]. The difference in pathogenicity may be due to the passage history of Ck/Yamaguchi/04 since the virus obtained from the National Institute of Animal Health (Japan) was propagated twice in embryonated chicken eggs before the present animal experiments. The pathogenicity of the H5N1 viruses isolated from humans in Hong Kong, 1997, was extremely high in mice [5, 7]. In the present study, more than half of the mice inoculated with Ck/Yamaguchi/04 survived the infection, indicating that the 50% mouse lethal dose was over $10^{8.0}$ EID_{50}. In conclusion, the pathogenicity of Ck/Yamaguchi/04 and Dk/Yokohama/03 in mice was much lower than that of the H5N1 viruses isolated from humans in Hong Kong, 1997.

Miniature pigs showed susceptibility to influenza virus, similarly to domestic pigs [3]. Miniature pigs were not susceptible either to Ck/Yamaguchi/04 or to Dk/Yokohama/03, but limited viral replication was observed in upper respiratory tissues in the miniature pigs inoculated with R(Dk/Mong-Dk/Mong). Therefore, the pigs may not play a major role in the maintenance and spread of Ck/Yamaguchi/04 and Dk/Yokohama/03. In contrast, H5N1 viruses isolated in 1997 from a boy (Hong Kong/156/97) and chicken (Ck/Hong Kong/258/97) replicated in pigs, although transmission through contact was not detected [24]. These results suggest that the susceptibility of pigs to avian influenza viruses has no relation to the pathogenicity of the strains in chickens or their subtypes, indicating that possible factors involved in host range restriction may be located in some gene segment(s) other than the HA gene [11, 23].

In conclusion, Ck/Yamaguchi/04 is highly pathogenic to birds and cause systemic infection, including brain. The results indicate that the susceptibility of pigs to this HPAI virus is very low, and that the possibility of genetic reassortments with this HPAI virus in pigs is not a concern.

Acknowledgments

We extend special thanks to the National Institute of Animal Health (Ibaraki, Japan) and the Animal Quarantine Service (Kanagawa, Japan) for providing the A/chicken/Yamaguchi/7/04

(H5N1) and A/duck/Yokohama/aq-10/03 (H5N1) influenza viruses, respectively. We also thank Dr. A. S. Mweene for discussing the contents of and English in this paper.

References

1. Alexander DJ (2000) A review of avian influenza in different bird species. Vet Microbiol 74: 3–13
2. Alexander DJ (2004) Highly pathogenic avian influenza. In: Manual of diagnostic tests and vaccines for terrestial animals (mammals, birds and bees), 5th edn, vol. 1. OIE, Paris, pp 258–269
3. Bai GR, Sakoda Y, Mweene AS, Kishida N, Yamada T, Minakawa H, Kida H (2005) Evaluation of the ESPLINE® INFLUENZA A&B-N Kit for the diagnosis of avian and swine influenza. Microbiol Immunol 49: 1063–1067
4. Chen H, Smith GJD, Zhang SY, Qin K, Wang J, Li KS, Webster RG, Peris JSM, Guan Y (2005) H5N1 virus outbread in migratory waterfowl. Nature 436: 191–192
5. Dybing JK, Schultz-Cherry S, Swayne DE, Suarez DL, Perdue ML (2000) Distinct pathogenesis of hong-kong-origin H5N1 viruses in mice compared to that of other highly pathogenic H5 avian influenza viruses. J Virol 74: 1443–1450
6. Fouchier RA, Munster V, Wallensten A, Bestebroer TM, Herfst S, Smith D, Rimmelzwaan GF, Olsen B, Osterhaus AD (2005) Characterization of a novel influenza A virus hemagglutinin subtype (H16) obtained from black-headed gulls. J Virol 79: 2814–2822
7. Gao P, Watanabe S, Ito T, Goto H, Wells K, McGregor M, Cooley AJ, Kawaoka Y (1999) Biological heterogeneity, including systemic replication in mice, of H5N1 influenza A virus isolates from humans in Hong Kong. J Virol 73: 3184–3189
8. Horimoto T, Nakayama K, Smeekens SP, Kawaoka Y (1994) Proprotein-processing endoproteases PC6 and furin both activate hemagglutinin of virulent avian influenza viruses. J Virol 68: 6074–6078
9. Ito T, Okazaki K, Kawaoka Y, Takada A, Webster RG, Kida H (1995) Perpetuation of influenza A viruses in Alaskan waterfowl reservoirs. Arch Virol 140: 1163–1172
10. Kida H, Brown LE, Webster RG (1982) Biological activity of monoclonal antibodies to operationally defined antigenic regions on the hemagglutinin molecule of A/seal/Massachusetts/1/80 (H7N7) influenza virus. Virology 122: 38–47
11. Kida H, Ito T, Yasuda J, Shimizu Y, Itakura C, Shortridge KF, Kawaoka Y, Webster RG (1994) Potential for transmission of avian influenza viruses to pigs. J Gen Virol 75: 2183–2188
12. Kida H, Yanagawa R, Matsuoka Y (1980) Duck influenza lacking evidence of disease signs and immune response. Infect Immunol 30: 547–553
13. Kishida N, Sakoda Y, Isoda N, Matsuda K, Eto M, Sunaga Y, Umemura T, Kida H (2005) Pathogenicity of H5 influenza viruses for ducks. Arch Virol 150: 1383–1392
14. Klenk HD, Garten W (1994) Host cell ptotease controlling virus pathogenicity. Trends Microbiol 2: 39–43
15. Li KS, Guan Y, Wang J, Smith GJ, Xu KM, Duan L, Rahardjo AP, Puthavathana P, Buranathai C, Nguyen TD, Estoepangestie AT, Chaisingh A, Auewarakul P, Long HT, Hanh NT, Webby RJ, Poon LL, Chen H, Shortridge KF, Yuen KY, Webster RG, Peiris JS (2004) Genesis of a highly pathogenic and potentially pandemic H5N1 influenza virus in eastern Asia. Nature 430: 209–213
16. Liu J, Xiao H, Lei F, Zhu Q, Qin K, Zhang X-w, Zhang X-l, Zhao D, Wang G, Feng Y, Ma J, Liu W, Wang J, Gao GF (2005) Highly pathogenic H5N1 influenza virus infection in migratory birds. Science 309: 1206

17. Makarova NV, Ozaki H, Kida H, Webster RG, Perez DR (2003) Replication and transmission of influenza viruses in Japanese quail. Virology 310: 8–15
18. Mase M, Tsukamono K, Imada T, Imai K, Tanimura N, Nakamura K, Yamamoto Y, Hitomi T, Kira T, Nakai T, Kiso M, Horimoto T, Kawaoka Y, Yamaguchi S (2005) Characterization of H5N1 influenza A virus isolated during the 2003–2004 influenza outbreak in Japan. Virology 332: 167–176
19. Mase M, Eto M, Tanimura N, Imai K, Tsukamoto K, Horimoto T, Kawaoka Y, Yamaguchi S (2005) Isolation of a genotyopically unique H5N1 influenza virus from duck meat imported into Japan from China. Virology 339: 101–109
20. Nestorowicz A, Kawaoka Y, Bean WJ, Webster RG (1987) Molecular analysis of the hemagglutinin genes of Australian H7N7 influenza viruses: role of passerine birds in maintenance or transmission? Virology 160: 411–418
21. Perkins LE, Swayne DE (2002) Pathogenicity of a Hong Kong-origin H5N1 highly pathogenic avian influenza virus for emus, geese, ducks, and pigeons. Avian Dis 46: 53–63
22. Reed LJ, Muench H (1938) A simple method for estimating fifty percent endpoints. Am J Hyg 37: 493–497
23. Scholtissek C, Burger H, Kistner O, Shortridge KF (1985) The nucleoprotein as a possible major factor in determining host specificity of influenza H3N2 viruses. Virology 147: 187–294
24. Sims LD, Ellis TM, Liu KK, Dyrting K, Wong H, Peiris M, Guan Y, Shortridge KF (2003) Avian influenza in Hong Kong 1997–2002. Avian Dis 47: 832–838
25. Stieneke-Grover A, Vey M, Angliker H, Shaw E, Thomas G, Roberts C, Klenk HD, Garten W (1992) Influenza virus hemagglutinin with multibasic cleavage site is acticated by furin, a subtilisin like endoprotease. EMBO J 11: 2407–2414
26. Suarez DL, Perdue ML, Cox N, Rowe T, Bender C, Huang J, Swayne DE (1998) Comparisons of highly virulent H5N1 influenza A viruses isolated from humans and chickens from Hong Kong. J Virol 72: 6678–6688
27. Tumpey TM, Kapczynski DR, Swayne DE (2004) Comparative susceptibility of chickens and turkeys to avian influenza A H7N2 virus infection and protective efficacy of a commercial avian influenza H7N2 virus vaccine. Avian Dis 48: 167–176
28. Xu X, Subbarao K, Cox NJ, Guo Y (1999) Genetic Characterization of the pathogenic influenza A/Goose/Guangdong/1/96 (H5N1) virus: similarity of its hemagglutinin gene to those of H5N1 viruses from the 1997 outbreaks in Hong Kong. Virology 261: 15–19

Author's address: Prof. Hiroshi Kida, Laboratory of Microbiology, Department of Disease Control, Graduate School of Veterinary Medicine, Hokkaido University, Sapporo, Hokkaido 060-0818, Japan; e-mail: kida@vetmed.hokudai.ac.jp

医薬品
医薬部外品　研究報告　調査報告書
化粧品

別紙 3-10

識別番号・報告回数		回	報告日 年　月　日	第一報入手日 2006 年 6 月 2 日	新医薬品等の区分 該当なし	総合機構処理欄
一般的名称			研究報告の公表状況	The public health risk from highly pathogenic avian influenza viruses emerging in Europe with specific reference to type A/H5N1. European Centre for Disease prevention and Control, scientific advice, June 1, 2006	公表国 スウェーデン	
販売名（企業名）						

研究報告の概要	使用上の注意記載状況・その他参考事項等
欧州の病原性トリインフルエンザウイルス(以下，HPAIVs)，特にトリインフルエンザ A（H5N1）型（以下 A/H5N1）によるヒトの健康へのリスクを評価している。野鳥と家禽での HPAIVs の世界的な大発生は 1990 年代より増加していて，現在欧州に及んでいる。1997 年までヒトへの感染は非常に少数であった。しかし，1997 年にアジアでヒトでの致死率が高く(50%以上)，遺伝子学上安定した A/H5N1 ウイルス株が出現したため，世界の保健当局は大きな懸念を示した。ヒト感染の主要ルートは重度に感染したトリとの直接接触，または調理されていないトリ食品の摂食である。現時点で，アジアで数件のヒト間での感染症例が報告されている。疫学データによると，A/H5N1 は最初に発見された時点と変わらずヒトに適応していないため，現在のウイルス型によって流行が引き起こされることはほとんどないと示唆された。欧州では実際，トリとヒトは地理的に隔離されているためヒトの健康への影響は非常に低い。対照的に，アフリカ，南アジア，中東では理論上のリスクは存在する。 A/H5N1 暴露によるヒトへの影響は不明確であるが，大規模な家禽に対する予防接種が高リスクの国々で行われた。 H5N1 ウイルスのヒトへの感染拡大予防のため、ECDC(途上国間地域経済協力)は以下を奨励している 1．野鳥と家禽の頻繁な調査 2．獣医と医療サービスの密接な協力 3．高リスクの人々への適切な情報と監査	BYL-2006-0234 www.ecdc.eu.int/influenza/update_influenza.php

報告企業の意見	今後の対応
インフルエンザウイルスに対する弊社の血漿分画製剤の安全性は，菌株の種類に関わらずウイルス不活化工程により確保されている。	現時点で新たな安全対策上の措置を講じる必要は無いと考える。引き続き関連情報の収集に努める。

(3)

ecdc

EUROPEAN CENTRE FOR DISEASE PREVENTION AND CONTROL

TECHNICAL REPORT

ECDC SCIENTIFIC ADVICE

The Public Health Risk from Highly Pathogenic Avian Influenza Viruses Emerging in Europe with Specific Reference to type A/H5N1 Version June 1st 2006

www.ecdc.eu.int

The Public Health Risk from Highly Pathogenic Avian Influenza Viruses Emerging in Europe with Specific Reference to type A/H5N1

Interim ECDC Risk Assessment – Revision 20th May 2006
(previous versions from 19th October 2005 and 5 January 2006)

Preamble

The objective of this revised document is to further determine the risk to human health in Europe from highly pathogenic avian influenza viruses in birds and animals. Specifically the additional risk that arises from the recent emergence and extension of A/H5N1 viruses into the European Union and elsewhere in the world, and the changed biology of the viruses among wild and domestic birds. In addition the document identifies areas requiring additional scientific and public health work both as single pieces of work and for risk monitoring by ECDC and its partners. Given the rapidly developing epidemiology in Europe and elsewhere the document remains an interim assessment, that will be regularly updated. The document should be considered along with other relevant publications concerning Avian Influenza produced by ECDC and other authoritative bodies such as WHO, he European Commission, the FAO, OIE etc. ECDC's collection of documents on H5N1 - the H5N1 Portfolio – is available at /www.ecdc.eu.int/avian_influenza/index.php.

Weekly updates are published in ECDC's Influenza Surveillance and Risk Monitoring summaries at www.ecdc.eu.int/influenza/update_influenza.php

Comments and contributions to the document continue to be welcomed to influenza@ecdc.eu.int

Executive Summary

Outbreaks of highly pathogenic avian influenza (HPAI) viruses in domestic poultry have been increasing since the late 1990s and have affected poultry in Europe as elsewhere.

Essentially there are two forms of risk to human health from these viruses:
- direct infection of humans with the avian virus
- the emergence of a new pandemic strain of type A influenza.

The human health impact of HPAI epizootics was very small, and almost unnoticed, until 1997. Infections were generally minor and usually self-limiting. The appearance of A/H5N1 in Asia, changed this perspective when infection of humans with a high mortality rate was detected during an outbreak in Hong Kong in 1997 after a pause until around 2003 this pattern of infection has continued as huge epizootics extended across the domestic poultry populations of South East Asia. However considering the massive exposure in Asia from one HPAI type (A/H5N1) there have been very few human infections resulting from HPAI. In the over 200 reported human infections since 2004 mortality is around 57%.

Compared to before 2003 there have been some significant changes in the behaviour of the H5N1 viruses in birds. One strain has stabilised and has been spreading more easily through a range of bird species and this is the strain that has spread to the EU. In some countries outside the European Union that strain could become endemic in some domestic birds as it has seemingly done in certain wild species. In the Asia-Pacific region, Indonesia is currently the most active site of H5N1 transmission but, in general, reported activity appears reduced compared to similar periods of 2004 and 2005.

In contrast in Africa, the Middle East and South Asia there is some evidence of significant levels of infection in domestic poultry. There has to be caution here as surveillance is weak. Equally weak are the veterinary services which mean that the prospects for control may be bleak in the short term.

Mild and asymptomatic human H5N1 infection seems to be rare and the indications are that transmissibility of A/H5N1 to humans is still very low even for those directly exposed. Most infections continue to be acquired from exposures to high doses of virus from sick domestic poultry in household

settings. There is no evidence of transmission to humans from casual contact with infected wild birds. The clinical picture continues to be unusual for HPAIs in humans in that infections are usually severe and often affect not just the respiratory system. Though human outbreaks and cases have been occurring in settings where it has proved difficult to mount proper investigations and studies, there is no evidence of any recent significant change in the behaviour of the virus in humans. Human to human transmission occurs but there is no evidence that it has become more efficient, cluster size has not increased and the case fatality rate of human infection has remained extraordinarily high for a human influenza. There is thus, as yet no evidence that the viruses have become any better adapted to humans than they were nearly a decade ago. The few humans who are infected do so only when exposed to high doses or virus and are likely to become very ill. They are unlikely to be a major infection risk to their families and those providing care. However normal infection precautions must be taken and antivirals given to those most exposed, usually other household members. A notable feature has been the focus of infections in other family members which could indicate some genetic susceptibility.

Surveillance for human cases may be becoming harder where poultry immunization is widely but inevitably imperfectly practiced as the marker of local poultry deaths for human case detection is being lost. Declines in the number of sporadic human cases in some countries should therefore be interpreted cautiously. It is also unclear as yet if massive immunization poultry programmes increase or decrease the overall human population exposure to H5N1 viruses.

Despite the seeming lack of adaptation of H5N1 viruses a major caveat is the ability of influenza viruses to change, recombine, adapt and generally confound those attempting to control them. Though no H5 virus is known to have adapted to humans in the past it would be unwise to assume they cannot do so. Exposure of humans to H5N1 viruses must have increased considerably recently, for example in Africa. This does not necessarily change the pandemic potential of H5N1 viruses. However if through genetic recombination with human influenza or mutation the viruses can achieve any potential then they now must be more likely to do so sooner than when human exposure to H5N1 viruses was uncommon and localized.

The pattern of infection and disease seen in Asia for A/H5N1 may not be seen elsewhere and therefore close clinical and laboratory surveillance for and of

human cases in Europe will be vital. That said, the Asian pattern of A/H5N1 has to be expected for planning purposes: a group of influenza viruses of birds, poorly adapted to humans whom they find hard to infect except at high doses. They are dangerous as they are highly pathogenic in those few humans that do become infected, but then they generally do not transmit on to other humans.

In the European Union, where surveillance for HPAI in wild-life is strong, this has detected steady extension of the virus in wild birds, including non-migratory species, and some domestic poultry. This peaked in early 2006 seemingly following migrations and numbers have declined but not fallen to zero. The risk of reintroduction through further migrations is significant though very difficult to predict.

The direct risk to the health of people in Europe from A/H5N1 is very low, but not zero. Human outbreaks in Turkey, Iraq, Azerbaijan, Egypt and elsewhere have indicated the potential of infection of humans from sick domestic poultry and probably also wild birds. The risk is mostly concentrated in one human group, those with domestic or pet poultry. Such groups exist in most European countries and they need to be informed of the risk and how to protect themselves. These pose particular challenges in terms of protection and risk communication as some of these groups are poor, marginalized or simply difficult to reach. There are occupational groups at lower and mostly theoretical risk who should take precautions. For those people who have no contact with domestic or wild birds or their products the risk must be almost non existent.

Monitoring human H5N1 infections and other emerging influenza viruses is of crucial importance as probably it's only in the early phases of emergence of a pandemic that there is any hope of containment. Though such emergence could take place in the European Union that seems unlikely. Though there are places not far beyond the borders of Europe where this could occur as well as in Asia and Africa.

ECDC and its partners will continue to monitor the risk from H5N1 and other HPAIs actively. A surveillance system for human cases A/H5N1, compatible to that already in use by WHO elsewhere has been developed and adopted as part of surveillance of human influenza. In addition some specific pieces of work on immunisation with seasonal vaccines are recommended. Most crucial will be continuing and developing close working of those responsible for

animal and human health at all levels, proper risk communication and dissemination of factual scientific data to the public including those few people at risk of infection.

It does not follow from any of the above that the next pandemic will necessarily be due to H5N1 or another HPAI. Equally since it is not fully understood how pandemics arise it does not follow that the risk of a pandemic is actually any higher now than it was say a decade ago. Though there is more H5N1 in circulation it does not follow that there has been an overall increase worldwide of the influenza viruses (of all H types) whose genetic material has pandemic potential.

Though there has been no increase in the pandemic potential of H5N1 the likelihood that it might achieve any inherent potential in the near future may have risen. There are many good reasons why the momentum of pandemic preparations in EU countries and preparations for possible outbreaks of H5N1 in birds and some human cases should continue and intensify. One implication for those determining policy is that if they are convinced that preparation should be made for a pandemic based on an H5N1 virus there are now reasons for speeding up those preparations.

New considerations since January

- It has become apparent that a strain of the H5N1 viruses has been able to affect a wide range of birds' species and has adapted well to certain migratory birds being able to travel widely with them extending its geographical range. These viruses have shown considerable stability over time.
- The range of these H5N1 viruses has greatly extended from being mostly confined to East and South East Asia to Europe including wild birds in the EU, the Middle East and parts of Africa and South Asia.
- Should the stability of the current H5N1 strains be maintained, Europe will have to adjust to add A/H5N1 influenza as one of endemic or occasionally appearing zoonotic infections. With H5N1 infections being in wild birds in all of Europe that risk will inevitably seem closer to home to EU citizens.
- Many more people worldwide are going to come into contact with H5N1. This will be less so in Europe than elsewhere because poultry in the EU are mostly segregated from humans.
- Though this does not mean any change in the pandemic potential of H5N1, if such potential exists at all it must now be more likely to become evident sooner rather than later.
- Certain other animal species notably cats can become infected naturally and in artificial conditions may occasionally transmit on the infection to other cats though no human infections have resulted.

Unchanged considerations

- There has been no indication of a significant change of behaviour of H5N1 viruses in humans. They currently remain *"a group of influenza viruses of birds, poorly adapted to humans whom they find hard to infect except at high doses. They are dangerous as they are highly pathogenic in those few humans that do become infected, but then they generally do not transmit on to other humans."*

- Control of infection in poultry along with risk communication to those at risk and prompt response (case finding and management) to human cases remain the cornerstones of strategies for protecting human health from H5N1 viruses.
- Human to human transmission occurs but remains uncommon and there has been no increase in cluster size where human cases have occurred.
- A few human cases have been detected in most countries where outbreaks in domestic poultry have occurred.
- The groups at risk in Europe are the same as before with the most important group being people with domestic poultry.
- There is no risk of catching H5N1 from eating food that has been prepared properly.

1. Background – the Zoonotic Potential of Avian Influenza

Influenza is an infectious disease of certain animals including humans with mostly respiratory characteristics. It is a zoonosis, that is an infection originally of animals which has extended to infect humans.[1] It is caused by RNA viruses of the family *Orthomyxoviridae*. These viruses are unstable in their structure and are continuously evolving.* Some of the viruses are well adapted to humans which have become their hosts and are regarded as human influenza viruses.† While the human infection and resulting disease caused can be mild or even asymptomatic it can also be severe and sometimes lethal for all age groups. It can extend beyond the respiratory system and is especially dangerous in the elderly and those with underlying chronic medical conditions. The most significant sudden impacts of influenza viruses on humans are those arising from the influenza A pandemic strains. These are novel or re-emerging viruses to which a large proportion of the human population have little immunity. They are thought to emerge through genetic recombination of human viruses or through recombination of human and animal viruses or perhaps changes in an animal virus and its adaptation to humans.[2] When pandemics emerge they quickly sweep world-wide before settling down to dominate the less severe seasonal influenza epidemics seen each winter. Since 1918 three strains have arisen causing major pandemics each resulting in millions of deaths. These were: H1N1 (1918) with an estimated forty million deaths world wide‡, H2N2 (1957) and H3N2 (1968) both with estimated deaths of between one and four million. A lesser pandemic occurred in 1977 when an H1N1 strain emerged without major mortality and only partially replaced the H3N2 strain so that at present both H1N1 and H3N2 strains circulate currently along with less pathogenic influenza B strains.[2]

The natural reservoir of influenza A strains is a diverse pool of viruses among aquatic wild bird populations, so called avian influenza (AI) viruses. These viruses are well adapted to many aquatic bird species, less so to other bird species while most are not at all adapted to humans and other mammals.[1]

---

* Influenza A viruses are classically characterized according to the serologic reaction to the surface glycoproteins into sixteen hemagglutinin subtypes (H1-16) and nine neuraminidase subtypes (N1-9). Not all potential combinations exist and of the 16 H types known, only subtypes H1, H2, H3, H5, H7, H9 and H10 seem capable of infecting humans.

† The families of influenza A viruses that are well adapted to humans are mostly in the subtypes H1, H2 and H3.

‡ The 1918 pandemic is considered exceptional in its high pathogencity and it particularly affecting young adults.

Adaptation means the viruses' ability to infect a host, reproduce and be transmitted onto fresh hosts. The AI viruses are divided on the basis of their impact on birds into those of high and low pathogenicity avian influenza (hence HPAI and LPAI) mostly on the basis of their biological characteristics. Highly pathogenic avian influenza (HPAI) viruses are those that when injected into chickens cause a high mortality (over 75%). All are H5 or H7 influenza A subtypes and an alternative criteria not requiring biological testing is any H5 or H7 virus with a haemaglutinin proteolytic cleavage site compatible with an HPAI virus.[3§]

2. Risk to Human Health from Avian Influenza Viruses - Principles

Essentially there are two mechanisms of risks to the physical health of humans from avian influenza viruses,

Direct or indirect[‡] infection causing disease and sometimes death.[4]

Pandemic Potential from the potential for the emergence of new pandemic strains either directly from avian viruses, or from their recombination of their genetic material (RNA) with RNA from human or other animal viruses.[2]

To realise any inherent genetic potential to cause a pandemic an influenza virus has to have three attributes. It has to be able to infect humans, to produce disease and most crucially to be efficiently transmitted from one human to another. Whilst HPAI infections as a whole carry a somewhat higher risk of producing infection in humans through direct or indirect infection it does not follow that because avian influenza is 'highly pathogenic' for birds that it has any greater risk of forming or contributing to a pandemic virus for humans.

---

§ On occasions in the last decade HPAI viruses have arisen that have a cleavage site that has not been seen before. Hence, the in vivo pathogenicity index is still necessary to judge an AI virus highly pathogenic if it has a cleavage site that has never been associated before with HPAI.

‡ Indirect meaning from the environment of fomites whereby live virus is deposited and survives for a short while. E.g. on hands, a towel or another surface and then is transferred to a human e.g. by shaking hands or sharing towels.

3. The History and Development of Highly Pathogenic Avian Influenza

3.1 Infections among Animals

Between 1959 and 2005 twenty-four HPAI epizootics (epidemics in animals) have been documented worldwide. These are all due to the A/H5 and A/H7 groups with types A/H5/N1-N3, N8, N9 and A/H7/N1, N3, N4, N7 respectively.[4,5] Many million birds have died in these epizootics either directly from the infection or from culling undertaken to control the infection. Outbreaks have sometime been due to the introduction of HPAI from wild birds. Equally some low pathogenicity A/H5 and A/H7 strains have mutated to become HPAI viruses following circulation among domestic poultry.[6]

Since 2000 there have been more and larger outbreaks of HPAI in poultry.[4,5] The reason for this is unknown and the subject of speculation. Both large and small outbreaks have taken place in Europe. Notable very large outbreaks have occurred in densely populated commercial bird populations such as in Italy in 1999 (type A/H7N1)[7], the Netherlands, Belgium and Germany in 2003 (type A/H7N7) and Canada in 2004 (H7N3).[8,9,10,11]

3.2 Human Infections due to Avian Influenza

The first documented human infection with an avian influenza (A/H7N7) goes back to 1959.[4] Cases occur in association with both large and small outbreaks in birds but not all animal influenza infections in humans have come from birds. For example in the late 1970s some workers dealing with infections in seals developed eye infections (conjunctivitis).[12] However infected birds seem to have been the major source of risk to humans.[4] There have been human infections with both low pathogenicity and high pathogenicity strains. Human infections with LPAI are recorded only occasionally but these have all been with minor self-limiting illnesses so it may be that they are under-recognised. For example there was one infection of a woman in the UK in 1996 receiving an eye infection from her domestic poultry which had mixed with wild birds and in 2006 a single case in a person seemingly exposed occupationally.[13,14] No LPAI virus has been reported connected with severe disease or death in a human.[4] In contrast the HPAI outbreaks in birds have resulted in at least 217 human cases and 123 deaths (a case fatality rate near

57%)**. Apart from a single fatality attributed to A/H7N7 virus strain during the Netherlands / Belgium / German outbreak all the deaths and most of the severe disease have been due to A/H5N1 (Figure1).[4,5,15] Mild human infections have been reported in a number of outbreaks, for example in Italy and Western Canada.[7,9,16] In the Netherlands, during the epizootic of HPAI with a different virus (A/H7N7), up to 64% of persons exposed to the virus showed a serological response consistent with infection.[17]

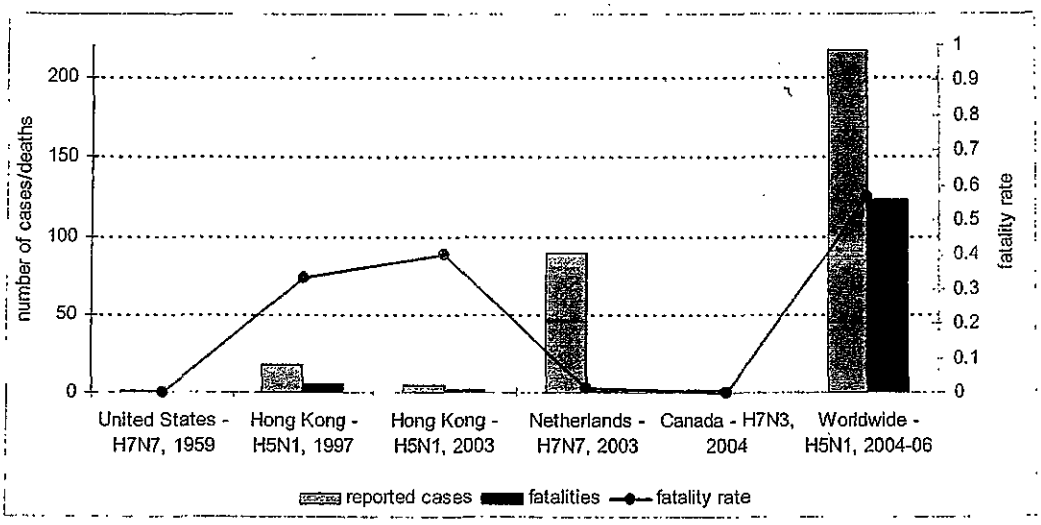


Figure 1. Reported human cases of infection and deaths with HPAI virus strains, 1959 – 2006.

** A/H5N1 data infections meeting WHO criteria as of 19 May 2006
http://www.who.int/csr/disease/avian_influenza/country/cases_table_2006_05_19/en/index.html

4. A/H5N1 - an unusual HPAI [††]

4.1 The Emergence of H5N1

In 1997 a series of poultry outbreaks of highly pathogenic avian influenza occurred in Hong Kong. An A/H5N1 strain was isolated both from chicken and humans (18 human cases, 6 fatalities). This included the first human to human transmission and the first occupational infection of a health care worker.[18,19,20,21] The outbreak was contained by the rapid culling of infected and at risk poultry and biosecurity measures. The virus strain is thought to have been circulating in Mainland China before 1997.[4,22,23]

These A/H5N1 viruses are a group of evolving viruses forming distinct strains some of which have achieved a degree of genetic stability. They have an ability to infect a surprisingly wide range of bird and even some animal species (for example the cat family with some cat-to-cat transmission in artificial circumstances).[22,24,25] This ability to transmit from mammal to mammal has raised concern of public health officials.[26]

The A/H5N1 viruses were not detected again until they reappeared in Hong Kong in February 2003 in humans (5 cases, 2 fatalities).[23] The infection was again controlled in poultry through vigorous culling, biosecurity measures and poultry vaccination. However this was the prelude to a vast expansion of the infection in the poultry populations in the Far East in Vietnam, Thailand, Cambodia and Indonesia and beyond.[23] The drivers for this rapid dissemination are unclear but commercial movements of chicks, birds and their products are as likely as dissemination through wild birds. By the end of 2005, over 140 human cases with a fatality rate close to 50% had been reported to the World Health Organization (WHO) from five counties.[23] It was on this basis of this spread and the possibility of A/H5N1 further adapting to humans that WHO raised its global influenza alert to Pre-Pandemic Alert Phase 3 in 2005.[27‡‡] To date that further adaptation has not been observed, specifically clusters of human H5N1 infection have not expanded in size as would be the case if there had been increases in human to human transmission.[28] However the potential may remain. It is thought by some that

†† The on-going history of A/H5N1 in animals and humans is well described in tabular form in the *WHO Time Line* available at http://www.who.int/csr/disease/avian_influenza/timeline.pdf

‡‡ In 2004 WHO changed its Pandemic Scale to a Six Point measure with three Pre-Pandemic Phases. Phase Three is when a novel influenza A virus has appeared can infect humans and cause disease, occasionally transmits from human to humans but has yet to show efficient person to person transmission.

a particular risk of pandemic emergence arises through recombination with circulating well-adapted (human) viruses through dual infections in humans and other mammals to produce a pandemic strain.[29,30]

By early 2005 H5N1 was causing special concern because it was becoming widely distributed in East and South East Asia.[23] The next major development, the dissemination of a particularly stable strain well adapted to birds beyond East and South East Asia seems to have started from an important natural event in China. This was at Qinghai Lake in West Central China where in April 2005 there was a large die-off of wild birds affecting an unusually diverse range of bird species.[23,31] Subsequent bird outbreaks across Asia, Europe and Africa have mostly been of the same strain as that observed at Qinghai, an unusual genetic stability for an avian influenza. When compared to earlier strains the virus has shown no diminution in its pathogenicity for chickens or humans.[23,32,33,34,35] The same virus then started to be seen in well migratory and non-migratory wildfowl and showed some indication of being somewhat more persistent in the environment though peer-review publications confirming this latter point have yet to appear.[36] Hence it became apparent in late 2005 that some H5N1 viruses could travel long distances quickly with wild birds as the vectors.[23,37]

Single cases or outbreaks in wild birds were subsequently detected in early 2006 in many European countries where wild bird surveillance was already in place.[23,38] Outbreaks were also detected in Middle Eastern, African and South Asian countries.[23] In these settings wild bird surveillance is unusual and so detection has usually taken place when either a domestic or commercial flock has been affected or when human deaths occur.[23]

The emphasis on wild birds does not mean that they have been the only source of dissemination though nobody could deny their role.[29,39,40] There are other important routes of local spread, notably through commercial practices and poor biosecurity (e.g. movements of infected poultry and people and vehicles with contaminated fomites). Control of these latter factors are crucial for protection of animals and humans at the local level.[29,40]

4.2 Increased Human Exposure to H5N1 2005-6 – the Implications

The range of the stable strain of H5N1 has extended considerably in 2005-6. Outbreaks in domestic poultry have expanded in some Regions where veterinary services are weaker than in the European Union. Therefore in

those places the likelihood of control of the infection is low and the numbers of people potentially exposed to H5N1 though domestic flocks has increased dramatically. That is in the Middle East, Africa and South Asia. This means that there will be more people who are at direct risk of H5N1 infection.[30] Equally there are many more governments that are needing to prepare for this eventuality. Though there may not have been any change in the pandemic potential the likelihood of any potential manifesting in the near future must have increased. This will be discussed in Section 5.

4.3 Poultry Immunisation to protect against H5N1

At least three countries, China, Indonesia and Viet Nam are undertaking large-scale poultry vaccination programs against H5N1, seemingly as medium term strategies and with the objective of reducing disease and the need for culling in poultry. The impact of these strategies on human risk of infection and disease is unclear. If poultry immunization is efficient and well monitored it could reduce the population burden of H5N1 in poultry and hence the risk for humans.[40] Equally however if it leads to the silent circulation of H5N1 in poultry it could actually increase the threat to humans in those countries and the risk of co-infection with other influenzas. The closely studied programme in Viet Nam is perhaps most likely to reveal which of these alternatives is realized. One unintended effect of these programmes is that they may make surveillance for single cases and small clusters of human H5N1 more difficult. Outbreaks in poultry can become 'silent' and the marker of die-offs of domestic flocks could be lost when deciding which human pneumonias to investigate. Falling numbers of reported human cases in countries practicing large scale poultry immunization may therefore be misleading.

4.4 The Extension of A/H5N1 into Europe

H5N1 extended into Europe in wild birds in early 2006 (Figure 2) with outbreaks or single cases in 13 out of 25 EU countries. Prior to 2006 large scale wild bird surveillance and surveys had produced results that were entirely negative for H5N1. The outbreaks have been instructive. Almost all the H5N1 that has been seen in the European Union has been in wild birds with only a handful of outbreaks in commercial poultry.[38] This reflects generally high levels of biosecurity in the European Union in the commercial sectors. The presentations have sometimes been subtle with relatively few bird deaths, notable odd neurological behaviours and no striking die-off of poultry. This raises the possibility that the virus could spread to other areas

and not be immediately apparent. Die-offs in commercial flocks due to HPAI are unlikely to go unnoticed in Europe. However it is acknowledged that more could be known about the presence of HPAI in the wild bird population and especially migratory birds.[41] Commercial flocks of poultry in the European Union are on the whole more separated from wild birds than those in Asia and Africa and so are less likely to act as sentinels. After the pulse of H5N1 in the early spring of 2006 numbers of H5N1 wild bird cases are diminishing but has not gone away as shown by outbreaks in commercial and domestic poultry along the Danube (Romania) and in the Baltic (Denmark) (Figure 2). The threat may return later through further migrations. There is agreement that controlling H5N1 in wild birds is impossible and should the stability of the current H5N1 strains be maintained Europe may simply have to adjust to add A/H5N1 influenza as one of endemic or occasionally appearing zoonotic infections: Guidelines to that effect for human health have been developed by ECDC.[42]

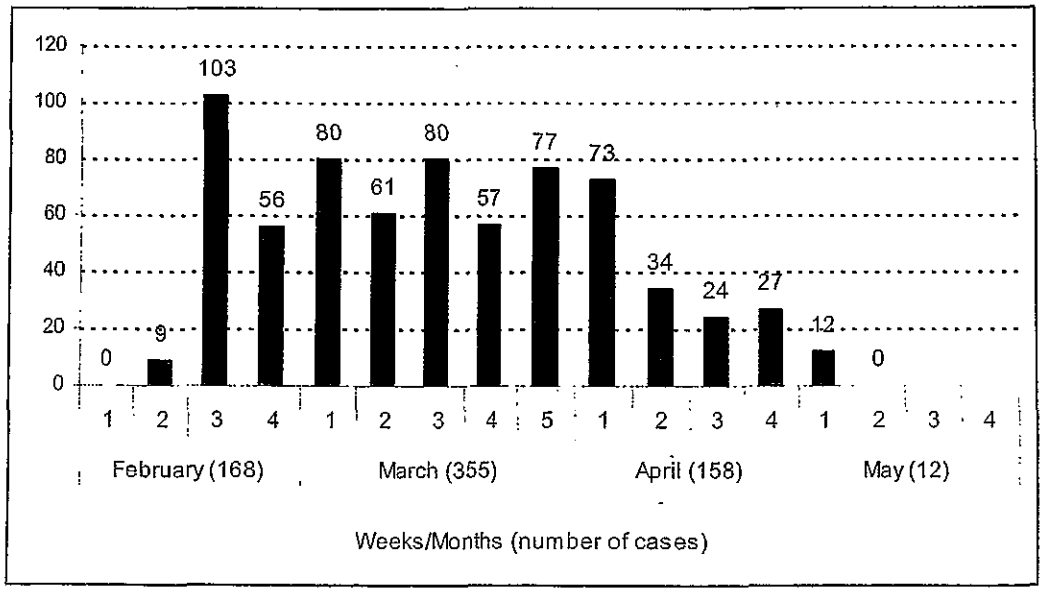


Figure 2. Highly Pathogenic Avian Influenza (H5N1) reported in the European Union through the Animal Disease Notification System February to May 2006 Source DG Sanco; http://europa.eu.int/comm/food/animal/diseases/adns/index_en.htm

4.5 Human A/H5N1 Cases - An Unusual Clinical and Epidemiological Profile

The only multi-country review of the clinical pattern of A/H5N1 in humans to date found that the infection and disease pattern differed significantly from any other human infections with HPAI.[30] Whilst it is certain that human cases have gone unrecognised and unreported, serological studies around cases to date have failed to identify mild or asymptomatic cases.[30] These serological studies have been criticized for being small scale and incompletely published. Also it has been suggested that the methods applied may only have the ability to detect serological responses in heavily ill hospitalized patients. However these findings are consistent with other results indicating that the H5N1 viruses are yet poorly adapted to humans.[23, 28]

A/H5N1 viruses do not transmit easily from birds to humans but when they do infect humans they cause severe disease.[30] It seems even less able to transmit on from human to human, which is typical of other poorly adapted zoonoses. [43,44,45] This combination of high pathogenicity and only occasional person to person transmission has changed little since the first observed infections in Hong Kong in 1997.[19,20,23]

4.6 Human Risk Groups and Risks of Transmission in Europe

See Table - Human Risk Groups in Europe (Annex 1)

While the routes of entry of H5N1 into humans remain poorly understood epidemiological data and the principles that follow from H5N1 being a virus as yet poorly adapted to humans indicate that the chances of humans becoming infected with an HPAI virus are small.[23] Equally the opportunity for transmission are confined to specific circumstances.
Human exposure to AI viruses occurs through contact with infected tissues, excretions, and secretions of infected birds, especially faeces and respiratory secretions.[30] The avian influenza viruses could seemingly be transmitted through various media: inhalation of contaminated dust, inhalation of fine water droplets, aerosols, hand-to-mucous membrane transfer of infected faeces or respiratory secretions and theoretically, mucous membrane exposure through consumption of raw or undercooked blood, organs or meat.[30] In general however, human cases have been principally related to close direct contact with high doses of virus from live or dead infected poultry

or occasionally wild birds.[46] Transmission probability is thought to be linked both to virus and host factors. Current efforts (genomic approaches, animal models, recombination approaches) are being undertaken in order to determine which characteristics allow viruses to infect humans.[30] However even though many millions of people in East and South East Asia have been directly exposed to H5N1 virus, only a very small percentage of them have become infected or ill.

Determining the exact routes of human infection and their risk factors has been beset with problems. Detailed field investigations have been rare and those with serological support even rarer.[30,46,47] Also in most cases there are multiple exposures and it is very difficult to determine if a person was infected by direct exposure to poultry, fomites, contaminated food or person to person transmission. There are a few case reports with seemingly reported unusual transmission (e.g. associated with bathing or consuming uncooked blood).[30,48] However further investigations of these have usually revealed multiple exposure and not evidence that the water or food was actually contaminated (R. Brown, WHO Vietnam, personal communication). Almost all of the A/H5N1 cases in Asia have been most closely associated with direct exposure to live or dead infected poultry.[30] Some cases suggest exposure only to raw poultry products. The handling and consumption of raw or undercooked products could be a source of human infection. This suggests there may be a need for a model for enteric transmission and mucous membrane exposure in addition to the usual respiratory models.

It has been suggested that these findings could have implications for Europe from environmental exposure to humans for example where wild migratory birds gather, for example at and around lakes. Certainly some studies of environmental contamination with HPAI where people and wild birds co-exist would be justifiable and risk assessments have been undertaken or are underway.[49,50] However it needs to be remembered that H5N1 remains poorly adapted to humans and that the greater risk for human infection (Table - Risk Group 1) is direct exposure to poultry raised or kept outdoors and those who have direct close contact with wild birds.[46] Poultry are highly susceptible to A/H5N1 Asian viruses and the expressed virus load grows to very high titres making the probability of exposure, infection and amplification and human infections greater through contact with outdoor-reared domestic poultry than indoor commercial or industrial poultry where biosecurity and worker protection is generally higher. There are also those who may theoretically be at risk though exposure (Table – Risk Group 2) but among

whom clinical infections have been very rare even in the Far East where exposure has been considerable.[23,30]

Three striking epidemiological features of human A/H5N1 infections have been

- how few infections have taken place considering the massive exposure to humans,
- the focus of infections in small household clusters involving family members
- the almost total absence of infections among those controlling the disease or caring for infected persons.

It has been suggested that a genetic susceptibility may partially explain some of these observations. Some comments have been made that children are more at risk (though the age structure of the human cases in the Far East is close to that of the population living in close proximity with poultry). This contrasts with the experience with the other HPAIs where those working to control the disease have been more at risk.[14,15,16,17]

This has implications for Europe as there is a risk, albeit very small to those who live closely with poultry and will probably not be so used to biosecurity considerations as those in the commercial farming sector – so called 'Sector 4' poultry owners, those with backyard or hobby poultry. This is especially so where those poultry may mix with migratory wild birds.

4.7 Risk to humans from Wild Birds

The experience from Azerbaijan indicate that there are rare circumstances where wild birds can pose a risk, for example if people attempt to handle and defeather sick or dead birds without taking precautions.[46] The public will be concerned but they need only follow simple measures already specified by European authorities and WHO such as not handling birds found dead and avoiding unnecessary contact with live birds when A/H5N1 has been shown to be present in a country.[42,46]

Finally however for the vast majority of people in Europe who do not have any of the above contact there can be hardly any risk at all of acquiring H5N1 infection while it remains in its present poorly adapted form.

Since unlike Asia, Africa and the Middle East most of the European Union's poultry flocks are segregated from humans the population risk is low. Those

working to control outbreaks of H5N1 are an obvious risk group. Good guidance for protection of this group already exists from the ECDC and also from other international and national sources in Europe and elsewhere.[51,52,53]

4.8 Risk from Food

Acquisition through food is a theoretical risk and has been demonstrated in the field and experimentally with tigers acquiring infection in Thailand from eating raw chicken and artificially infected cats.[24,25] However since cooking destroys the virus it should only be people consuming raw poultry products that would be at risk in Europe and there is already standard guidance to avoid such products including eggs.[54]

4.9 Identifying and Communicating with the Groups at Highest Risk

Because of the potential entry of the virus from migratory birds away from commercial flocks, the humans more at risk may be those with small flocks and a few backyard poultry (chickens, turkeys, ducks etc) and they also require guidance based on what has already been developed by WHO and UNICEF.[55,56] It is especially important to establish where there are such groups in Europe who are living with more intimate contact with domestic poultry and perhaps near migration sites. These groups can be hard to define and reach (e.g. families in poor circumstances without access to electronic communication). Those most at risk may be women who care for domestic poultry and children who play with them. However the extremely low transmissibility of A/H5N1 to people living like this observed in Asia is reassuring.

4.10 Preventing infection of Humans by H5N1 Viruses.

There is no single strategy that will uniformly prevent human infection with HPAI viruses though the most important strategy is control in poultry, the most likely way that people will be exposed. Three approaches seem sensible and have been supported internationally by WHO, OIE and FAO [29,40]

i. **Control the infection in birds which people will come into contact with – usually domestic poultry.**

ii. **Community mobilisation and education to reduce risk of human exposure to infected birds**

iii. **Case finding, surveillance, laboratory confirmation, treatment, patient isolation and infection control** Bearing in mind he people at highest risk are those living with other cases of H5N1 and after that people living intimately with domestic poultry

Most people in Europe will not be at risk (Table Risk Groups) though the potential widespread dissemination in the environment means that certain sensible precautions should be taken universally, most of these are around good general hygiene and should be being applied already and that is the basis of ECDC's advice to people living where H5N1 has been found.[42] The little risk that exists is mostly in groups that come into direct contact with birds. These groups need to take certain special precautions.

4.11 The importance of small household clusters – Person to Person Transmission

One paradox arising from the human data is that while the risk of H5N1 in any individual is very low once a case appears the risk of cases other household members rises considerably. There have been many small household clusters in China, South East Asia, Turkey, Iraq and Azerbaijan. Hence the emphasis on case finding and then early treatment of other household members in public health guidance.[57].

This observation has been misinterpreted as implying there is more person to person transmission than appreciated. The cause of these small clusters is unclear. They include shared exposure, some genetic susceptibility as well as person to person transmission. Occasional transmissions to very close contacts have been seen since 1997 but remain rare.[23,30] Those who have become infected were generally blood relatives providing care at home. Apart from one case there have been no onward transmissions to those providing care in a health setting and taking normal precautions.[23] Probably the most important observation is that the clusters are no bigger now in 2006 than they were in 1997 in contrast to what would have occurred should the virus have adapted to humans and become more transmissible.[23,28] It that occurs it which would be an indication that the world was entering WHO's Phase 4 or

Phase 5 of a pandemic at which point there would probably only be a single opportunity to contain the pandemic through early containment.[58]

5. The Potential for generation of a pandemic strain from H5N1.

A/H5N1 viruses have been circulating with occasional human exposures for nearly a decade. While the infection can infect and cause disease no change in behaviour of the virus in humans have been detected (in stark contrast to all the changes in birds) and no pandemic strain has appeared.[23] There must be some restriction on widespread transmission in humans of the H5N1 virus since the AI virus strains that infect humans seems to be limited to a restricted genotype and there have been few infections, even though millions of exposures and very close contact is required to infect other humans. Hence most evidence indicates a difficult adaptation process for A/H5N1 viruses among humans.

An enduring concern is that a "normal" seasonal flu virus will infect an H5N1-infected human, the two viruses will recombine and a new efficient H5N1 strain will emerge.[2,59] Equally there might be recombination with another animal influenza or the H5N1 could simply mutate to form a pandemic strain.[2] Previously it was thought that due to the low number of H5N1 infected humans, this would be statistically unlikely though the risk might increase as the epizootic continues. Given that normal human influenza has been circulating world wide, including in Asia, the extension of A/H5N1 to birds in Europe, Africa and South Asia must have increased the numbers of people potentially exposed to H5N1 and human influenzas. At the same time it is unknown whether poultry immunization in the Far East will decrease or increase human exposure.

The reduced amounts of contacts between infected birds and humans in European countries compared to elsewhere makes it unlikely that Europe will be the starting point for an H5N1 pandemic though there are countries on the edge of the European Union to the East and South where those conditions exist and where WHO's Early Containment Strategy might be needed.[58]

The extension of range does not mean that there has been any change in the pandemic potential of H5N1 itself.[30] However the increased exposure of humans to H5N1 means that if this virus does have pandemic potential that potential must be more likely to be expressed in the near future than it was previously. Conversely if H5N1 shows no ability to adapt better to humans despite such exposure confidence is likely to increase that its genetic pandemic potential is low. One implication for those determining policy is that if they are convinced that preparation should be made for a pandemic

based on an H5N1 virus there are now good reasons for speeding up those preparations.

It does not follow from any of the above that the next pandemic will necessarily be due to H5N1 or another HPAI. Equally since it is not fully understood how pandemics arise it does not follow that the risk of a pandemic is actually any higher now than it was say a decade ago. Though there is more H5N1 in circulation it does not follow that there has been an overall increase worldwide of the influenza viruses (of all H types) whose genetic material has pandemic potential.

However certainly there is absolutely no room for complacency. The first pandemic of the 20th century is thought to have emerged at least in part from an avian influenza either in Europe or North America.[60] It has been suggested that other mammals may act as the 'vessel' for dual infection and recombination.[2]

There are reasons for recommending extending seasonal influenza vaccination to wider groups. It has been suggested to include those involved in control measures when seasonal influenza is circulating. This is already a standard recommendation by one of WHO's Regions.[53] The case for immunisation of the wider number of people in Europe who live with domestic poultry either in commercial farms or with just a few chickens in the backyard is a much more difficult decision and requires a measured scientific public health view. A major consideration will be the difficulty of identifying those at risk. It probably will be preferable to further expand the use of seasonal vaccinations in the general population at least in the risk groups as recommended by WHO's Executive Board.[61] Given the need to expand European capacity for production of influenza vaccine there are other reasons why this will be desirable.

There are two crucial caveats to end this section. Firstly the ability of influenza viruses to adapt, change and surprise is well established. Just because the A/H5N1 viruses have been behaving in one way in Asia up to now that does not mean they cannot and will not and so become a greater threat to human health. Secondly it is important in this section to acknowledge that in focusing so much on HPAI in general and A/H5N1 in particular the next pandemic infection may actually arise from a low pathogenic strain, or the already human adapted A/H2N2 virus that caused a

previous pandemic because almost no community immunity exists against this virus anymore.

6. Final Considerations

6.1 The Need for Close Co-operation of Veterinary and Human Health Services

Much of the reduction of risk to humans from A/H5N1 will depend on the outcomes of veterinary control programs and how safely they are conducted.[29,40] While it would be difficult to justify large-scale public health expenditures in preventing a few sporadic human cases, it is justifiable to support expenditures to solve the problem in animals with such potentially significant public health implications as development of a pandemic. Increasing cooperation between the veterinary and human health agencies within Europe will be a crucial component to control avian influenza. Unified national and local planning is an imperative and is already part of the assessment exercises that ECDC, the European Commission and WHO Europe is assisting national authorities in making. Especially important will be unified approaches to any outbreaks of human H5N1 in the EU including vulnerable accession and candidate countries notably Romania.

6.2 The Importance of Risk Communication

Though strictly outside the remit of this paper it is impossible to ignore the evident confusion in the minds of the public between avian, seasonal and pandemic influenza. The perception of risk can be massive while as demonstrated above the actual risk to the individual from Avian Influenza is extremely low, even if they are exposed to infected poultry. Partially this confusion is understandable since avian influenza can lead onto pandemic influenza and the two issues are commonly tackled together in publications. However this is leading to disproportionate anxiety and needs to be addressed. Otherwise when the pandemic of 'bird flu' fails to materialize the case for preparing for the next pandemic will be undermined. Equally there will be unwarranted and disproportionate anxiety in the minds of the public and fear of harmless birds, both wild and domestic.

6.3 Adapting to H5N1

Though there is no sign that H5N1 is adapting to humans Europe needs to adapt to H5N1. The detection of H5N1 in wild birds in many European Union Countries and the seeming stability of the virus suggest that countries may

need to adjust to this being added to the current list of zoonoses present in animals that occasionally infect humans.

References

1 Neumann G, Kawaoka Y. Host range restriction and pathogenicity in the context of influenza pandemic. Emerg Infect Dis. 2006, June http://www.cdc.gov/nciod/EID/vol12no06/05-1336.htm
2 Nicholson KG, Wood JM, Zambon M. Influenza (seminar). Lancet 2003; 362: 1733-44.
3 Office International des Epizooties (OIE) Manual of Standards for diagnostic tests and vaccines 2004. Office International des Epizooties. Paris, France http://www.oie.int/eng/publicat/en_standards.htm
4 Perdue ML, Swayne DE. Public health risk from avian influenza viruses. Avian Diseases 2005; 49: 317-327
5 Capua I, Alexander DJ. Avian influenza: recent developments. Avian Pathology 2004; 33: 393-404.
6 Delay PD, Casey H, Tubiash HS. Comparative study of the fowl plague virus and a virus isolated from man. Public Health Rep. 1967; 82: 615-20.
7 Capua I. The 1999-2000 avian influenza (H7N1) epidemic in Italy. Vet. Res. Commun. 2003; 27: 123-127.
8 Elbers ARW, Fabri THF, de Vries TS et al. The highly pathogenic avian influenza A (H7N7) virus epidemic in the Netherlands in 2003. Avian Dis 2004; 48: 691-705
9 Koopmans M, Wilbrink B, Conyn M, et al. Transmission of H7N7 avian influenza A virus to human beings during a large outbreak in commercial poultry farms in the Netherlands. Lancet 2004; 363:587-593.
10 Bosman A ; Mulder YM ; Leeuw JRJ de Avian Flu Epidemic 2003: Public health consequences RIVM Report, Bilthoven, Netherlands 2004, http://www.rivm.nl/bibliotheek/rapporten/630940004.pdf
11 Tweed SA, Skowronski DM, David ST, Larder A, Petric M, Lees M, et al. Human illness from avian influenza H7N3, British Columbia. Emerg Infect Dis [serial on the Internet]. 2004 Dec Available from http://www.cdc.gov/ncidod/EID/vol10no12/04-0961.htm
12 Webster RG, Hinshaw VS, Bean WJ et al. Characterisation of an influenza A virus from seals. Virology 1981; 113: 712-724.
13 Kurtz J, Manvell J, Banks J. Avian influenza virus isolated from a woman with conjunctivitis. Lancet 1996; 348: 901-2.
14 Nguyen-Van-Tam J, Nair P, Acheson P et al Outbreak of low pathogenicity H7N3 avian influenza in UK, including associated case of human conjunctivitis. Eurosurveillance Weekly 2006; 11: 5 May 4th 2006 http://www.eurosurveillance.org/ew/2006/060504.asp#2
15 Fouchier RA, Schneeberger PM, Rozendaal FW, Broekman JM, Kemink SA, Munster V, Kuiken T, Rimmelzwaan GF, Schutten M, Van Doornum GJ, Koch G, Bosman A, Koopmans M, Osterhaus AD. Avian influenza A virus (H7N7) associated with human conjunctivitis and a fatal case of acute respiratory distress syndrome.
Proc Natl Acad Sci U S A. 2004 Feb 3;101(5):1356-61. Epub 2004 Jan 26.
16 . Puzelli S, Di Trani L, Fabiani C, Campitelli L, De Marco MA, Capua I, Aguilera JF, Zambon M, Donatelli I. Serological analysis of serum samples from humans exposed to avian H7 influenza viruses in Italy between 1999 and 2003. J Infect Dis. 2005 Oct 15;192(8):1318-22. Epub 2005 Sep 12
17 Meijer A, Bosman A, van de Kamp EEHM, et al. Measurement of antibodies to avian influenza virus A(H7N7) in humans by hemagglutination inhibition yest. J Virol Methods 2006;132:113-20
18 Sims ID, Ellis TM, Liu KK et al. Avian influenza in Hong Kong 1997-2002. Avian Dis 2003; 47: 832-838.
19 Mounts AW, Kwong H, Izurieta HS et al Case contol study of risk factors for avian influenza A (H5N1) Hong Kong 1997. J Infect Dis 1999; 180: 505-8
20 Centers for Disease Control and Prevention. Isolation of avian influenza A(H5N1) viruses from humans--Hong Kong, May-December 1997. MMWR Morb Mortal Wkly Rep. 1997; 46:1204-7.

21 Claas EC, Osterhaus AD, van Beek R, et al. Human influenza A H5N1 virus related to a highly pathogenic avian influenza virus. Lancet 1998; 351:472-7.
22 Xu X, Subbaro K, Cox NJ, Guo Y. Genetic characterisation of the pathogenic influenza A /goose/Guangdong/(H5N1) virus: similarity of its haemagglutinin gene to those of H5N1 viruses from the 1997 outbreaks in Hong Kong. Virology 1999; 261: 5-19.
23 World Health Organzation .H5N1 Timeline
http://www.who.int/csr/disease/avian_influenza/timeline.pdf
24 Rimmelzwaan GF, Van Riel D, Baars M et al Influenza A virus (H5N1) infection in cats causes systemic disease with potential novel routes of viral spread with and between hosts. American Journal of Pathology 2006; 168: 176-183
25 Infuenza Team ECDC. H5N1 infections in cats - public health implications Eurosurveillance 2006 11; 4 April 13th 2006 http://www.eurosurveillance.org/ew/2006/060413.asp#4
26 EU Statement of the Standing Committee on the Food Chain and Animal Health Brussels March 1st 2006
http://www.europa.eu.int/rapid/pressReleasesAction.do?reference=MEMO/06/104&format=HTML&aged=0)#uage=EN&guiLanguage=en
27 World Health Organization. WHO Global Influenza Preparedness Plan.
http://www.who.int/csr/resources/publications/influenza/WHO_CDS_CSR_GIP_2005_5.pdf
28 Anonymous Avian influenza – situation in Indonesia Update 12. May 18th 2006
http://www.who.int/csr/don/2006_05_18b/en/index.html
29 World Health Organization. Responding to the avian influenza pandemic threat. Recommended strategic actions. WHO, Geneva September 2005.
http://www.who.int/csr/resources/publications/influenza/WHO_CDS_CSR_GIP_2005_8/en/index.html
30 Writing Committee of the World Health Organization (WHO) Consultation on Human Influenza A/H5. Avian Influenza A (H5N1) Infection in Humans. NEJM 2005; 353:1374-1385.
31 World Health Organization Global Influenza Program Surveillance Network. Evolution of H5N1 avian influenza viruses in Asia. Emerg Infect Dis 2005; 11:1515-21
32 Li KS et al. Genesis of a highly pathogenic and potentially pandemic influenza virus in eastern Asia. Nature 2004; 430: 209–213.
33 Chen H et al. The evolution of H5N1 influenza viruses in ducks in southern China. Proc Natl Acad Sci USA 2004; 101: 10452–57.
34 Chen H et al. H5N1 virus outbreak in migratory waterfowl. Nature 2005; 436: 191–92.
35 Chen H et al. Establishment of multiple sublineages of H5N1 influenza virus in Asia: implications for pandemic control. Proc Natl Acad Sci USA 2006; 103: 2845–2850.
36 WHO Avian influenza: significance of mutations in the H5N1 virus. February 20th 2006
http://www.who.int/csr/2006_02_20/en/index.html
37 Avian Influenza Technical Task Force Update on the avian influenza situation (as of 01/09/2005 – Issue no. 33. Potential risk of HPAI spreading through wild water bird migration. Food and Agriculture Organization, September 2005.
38 European Union Animal Disease Notification System
http://europa.eu.int/comm/food/animal/diseases/adns/index_en.htm
39 European Food Safety Authority. Report: Opinion of the AHAW Panel related to animal health and welfare aspects of Avian Influenza September 2005
http://www.efsa.eu.int/science/ahaw/ahaw_opinions/1145_en.html
40 FAO and OIE A global strategy for the progressive control of highly pathogenic avian influenza. October 2005 http://www.fao.org/ag/againfo/subjects/documents/ai/HPAIGlobalStrategy31Oct05.pdf
41 European Commission. Standing Committee on the Food Chain and Animal Health statement October 14th 2005
42 ECDC Advice for people living or traveling where H5N1 has been detected
http://www.ecdc.eu.int/avian_influenza/Health_Advice.php

43 Hayden F, Croisier A. Transmission of Avian Influenza Viruses to and between Humans. J Infect Dis. 2005;192:1311-1314.
44 Ungchusak K, Auewarakul P, Dowell SF et al. Probable Person-to-Person Transmission of Avian Influenza A (H5N1). The NEJM 2005; 352:333-340
45 Liem NT, World Health Organization International Avian Influenza Investigation Team V, Lim W. Lack of H5N1 avian influenza transmission to hospital employees, Hanoi, 2004. Emerging Infectious Diseases 2005; 11(2):210-215
46 WHO Human avian influenza in Azerbaijan, February–March 2006 Weekly Epidemiological Record 2006 81: 183-188 http://www.who.int/wer/2006/wer8118/en/index.html
47 Areechokchai D, Jiraphongsa C, Laosiritaworn Y et al Investigation of Avian Influenza (H5N1) Outbreak in Humans, Thailand, 2004 MMWR April 28, 2006 / 55(SUP01);3-6
http://www.cdc.gov/mmwr/preview/mmwrhtml/su5501a2.htm
48 De Jong MD, Van Cam B, Qui P et al Fatal avian influenza in a child presenting with diarrhoea followed by coma. NEJM 205; 532: 686-91
49 ECDC Risk Assessment on Bathing Water (in press)
50 Schijven J, Teunis JVN, de Roda Husman AM. Quantatative risk assessment of avian influenza virus infection via water. RIVM Report 703719012/2005
51 World Health Organization.- Western Pacific Region Publications on Avian Influenza
http://www.wpro.who.int/health_topics/avian_influenza/publications.htm
52 European Centre for Disease Prevention and Control. Avian Influenza – Occupational Exposure.
http://www.ecdc.eu.int/avian_influenza/occupational_exposure.php
53 Health Protection Surveillance Centre, Health guidance for protection of persons involved in avian influenza outbreak control and eradication activities in Ireland. Dublin,Ireland, October 2005
http://www.ndsc.ie/A-Z/Respiratory/AvianInfluenza/Guidance/File,1372,en.pdf
54 Food Standards Agency. Egg, 2002
http://www.food.gov.uk/safereating/microbiology/eggs2002advice
55 United Nations Childrens Fund. Avian Influenza Update
http://www.unicef.org/ceecis/resources_4009.html
56 World Health Organization Advice for people living in areas affected by bird flu or avian influenza. WHO Western Pacific Region 2004 http://www.wpro.who.int/NR/rdonlyres/04FA6993-8CD1-4B72-ACB9-EB0EBD3D0CB1/0/Advice10022004rev08112004.pdf
57 WHO Rapid Advice Guidelines on pharmacological management of humans infected with avian influenza A (H5N1) virus May 2006
http://www.who.int/csr/disease/avian_influenza/guidelines/pharmamanagement/en/index.html
58 WHO Early Containment Document
http://www.who.int/csr/disease/avian_influenza/guidelines/fluprotocol_17.03.pdf
59 World Health Organization. Guidance for the use of seasonal influenza vaccine in humans at risk of H5N1 infection. WHO Western Pacific Region 2004
http://www.wpro.who.int/NR/rdonlyres/D89CEE14-3366-4D9E-B679-B43FAF0D368C/0/guidelinesfortheuseofseasonalinfluenzavaccine.pdf
60 Taubenburger et al Nature Vol 437 October 6th 2005 doi:10.1038/nature04320
http://www.nature.com/news/2005/051003/full/nature04230.html
61 Resolution of the Executive Board of WHO 2003. Prevention and Control of Influenza Pandemics and Annual Epidemics. Executive Board 111th Session Agenda item 5.8. January 2003

Annex 1. Table: Who is at risk of getting "Bird Flu" - Highly pathogenic H5N1 avian influenza?

Broadly speaking there are two types of Risk Groups:-

Group 1 - Low but Real Risk
The risk of infection is almost entirely confined to the small numbers of **people who have close and intense contact with sick H5N1 infected domestic poultry (chickens, ducks etc) or their droppings or sometimes wild birds. For example through having sick and H5N1 infected poultry in the house.**
Human cases have almost entirely been in this category.
In these circumstances **children** may be at higher risk than adults. This probably represents behavioral rather than constitutional susceptibility. In these setting children being more play with or look after poultry and are less likely to practice good persona hygiene than adults.

People traveling to countries where H5N1 is prevalent can sometimes enter this category if they are staying with families with domestic poultry.

The people who are **at highest risk of acquiring H5N1** are the very small number of **people living in the same household as cases of H5N1 in humans**. It is thought that this is through shared exposure. Though person to person transmission also occasionally happens. This is why early identification of human cases and early treatment of them and their household contacts is crucial.

Group 2 Theoretical Risk – Precautions Required
There are also those at theoretical risk who may be exposed to the virus and should take appropriate precautions. This includes the following where H5N1 may be present:

- Health care workers caring for those with H5N1 infection though there have been no cases in this group for nearly a decade the risk is there and preventive measures should be taken. A related group are those working in laboratories with H5N1 viruses
- Veterinarian and people involved in controlling outbreaks in birds (culling)
- People who work on industrial poultry farms,
- People who may have close contact with infected wild birds e.g. some ornithologists and hunters,
- People who deal with sewage which is contaminated with H5N1

For the majority of people who have no contacts with domestic or wild birds or their droppings, the risk of acquiring H5N1 is almost non existent.