資料D

感染症定期報告に関する今後の対応について

平成１６年度第５回
運営委員会確認事項
（平成１６年９月１７日）

１　基本的な方針

運営委員会に報告する資料においては、

(1) 文献報告は、同一報告に由来するものの重複を廃した一覧表を作成すること。

(2) ８月の運営委員会において、国内の輸血及び血漿分画製剤の使用した個別症例の感染症発生報告は、定期的にまとめた「感染症報告事例のまとめ」を運営委員会に提出する取扱いとされた。これにより、感染症定期報告に添付される過去の感染症発生症例報告よりも、直近の「感染症報告事例のまとめ」を主として利用することとすること。

２　具体的な方法

(1) 感染症定期報告の内容は、原則、すべて運営委員会委員に送付することとするが、次の資料概要を作成し、委員の資料の確認を効率的かつ効果的に行うことができるようにする。

① 研究報告は、同一文献による重複を廃した別紙のような形式の一覧表を作成し、当該一覧表に代表的なものの報告様式（別紙様式第２）及び該当文献を添付した「資料概要Ａ」を事務局が作成し、送付する。

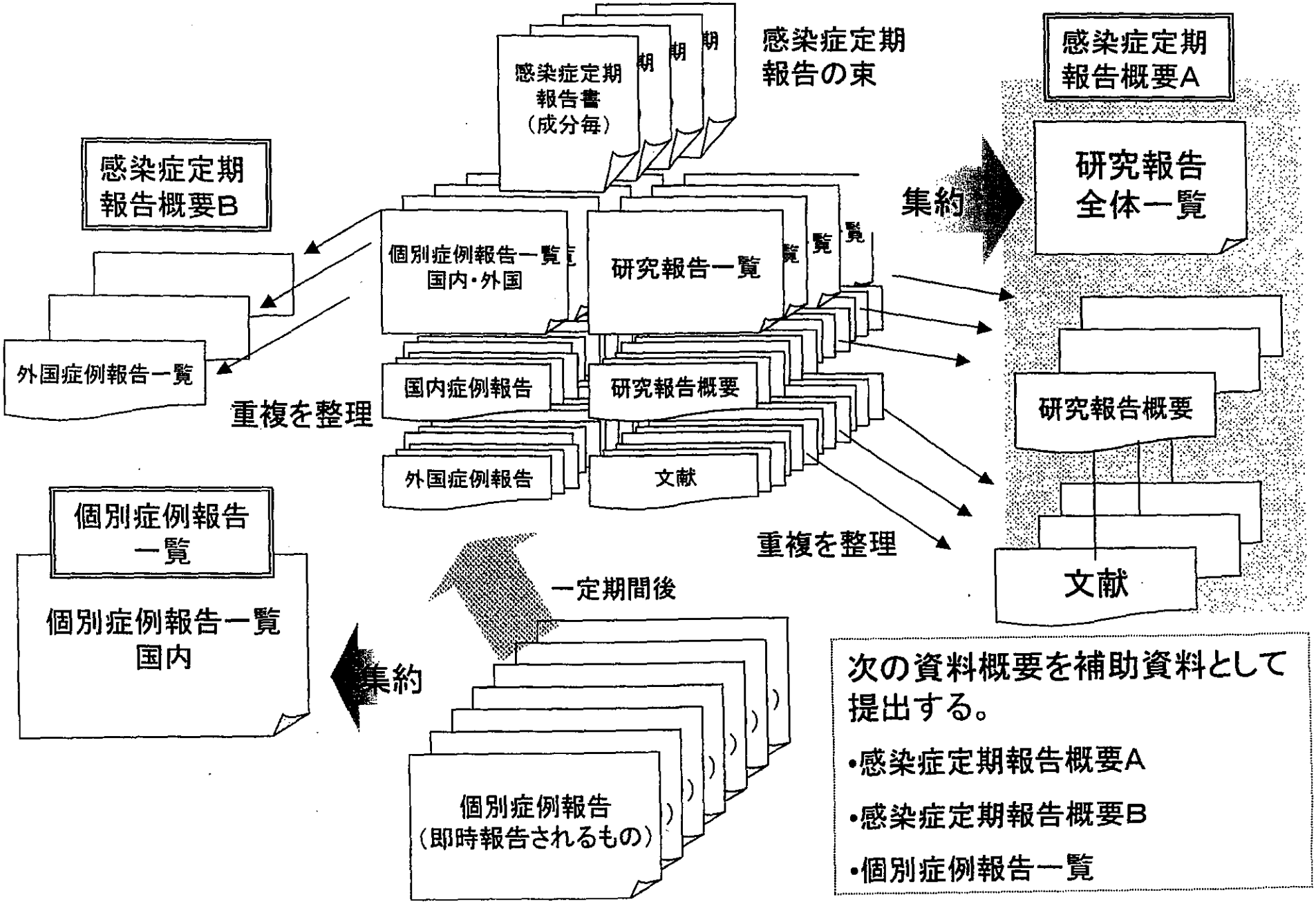
② 感染症発生症例報告のうち、発現国が「外国」の血漿分画製剤の使用による症例は、同一製品毎に報告期間を代表する感染症発生症例一覧（別紙様式第４）をまとめた「資料概要Ｂ」を事務局が作成し、送付する。

③ 感染症発生症例報告のうち、発現国が「国内」の輸血による症例及び血漿分画製剤の使用による感染症症例については、「感染症報告事例のまとめ」を提出することから、当該症例に係る「資料概要」は作成しないこととする。ただし、運営委員会委員から特段の議論が必要との指摘がなされたものについては、別途事務局が資料を作成する。

(2) 発現国が「外国」の感染症発生症例報告については、国内で使用しているロットと関係がないもの、使用時期が相当程度古いもの、因果関係についての詳細情報の入手が困難であるものが多く、必ずしも緊急性が高くないと考えられるものも少なくない。また、国内症例に比べて個別症例を分析・評価することが難しいものが多いため、緊急性があると考えられるものを除き、その安全対策への利用については、引き続き、検討を行う。

(3) 資料概要Ａ及びＢについては、平成１６年９月の運営委員会から試験的に作成し、以後「感染症定期報告について（目次）」資料は廃止することとする。

感染症定期報告・感染症個別症例報告の取り扱い



次の資料概要を補助資料として提出する。

- 感染症定期報告概要A
- 感染症定期報告概要B
- 個別症例報告一覧

感染症定期報告概要

（平成１８年１０月３１日）

平成１８年６月１日受理分以降

Ａ　研究報告概要
Ｂ　個別症例報告概要

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６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6月1日～平成18年8月31日）　　資料1

血対ID	受理日	感染症（PT）	出典	概要	
60062	2006/06/20	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。	
60056	2006/06/02	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年代から緩やかに始まり、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ことが示唆された。	1
60056	2006/06/02	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。	
60077	2006/07/10	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く、血液製剤によるHEV伝播の時期を示唆する結果が得られた。	2
60085	2006/07/24	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、シカなどの生肉、生レー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、ホルモンの喫食歴のある献血者は49,361人中13,835人（28.0%)で、その血液検体からHEV-RNAが5例検出された。平成17年1月1日から12月31日に、試行的HEV20プールNAT検査を行ったところ、295,442人中30人が陽性で、約1/10000の陽性率であった。	
60077	2006/07/10	E型肝炎	日本輸血学会誌 2006; 52: 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。HEV問診や抗体スクリーニングはHEV RNA陽性者の排除には有効ではない。	3
60085	2006/07/24	HIV	CDR Weekly 2006; 16(4) HIV/STIs Reports	英国におけるHIVおよびAIDSの四半期最新情報（2005年12月末までのデータ）。HIV診断総数は増加を続けており、2005年度は7,700例を越えると予想される。2004年から2005年のHIV診断数増加の大部分は男性と性交渉を持つ男性が占める。現在のところ2005年の新規のAIDS診断数は474例であった。1982年のサーベイランス開始以降の英国での累計はHIV診断数76,850例、AIDS診断数21,898例となった。	

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60056	2006/06/02	HIV	J Med Virol 2006; 78: 311-317	ドイツで初めてB/Gサブタイプ間組換え型ヒト免疫不全ウイルス1型（HIV-1)が同定された。このウイルスは、NucliSense HIV-1 QT assay (Organon Tecknika/bioMerieux)では検出不能であり、Monitor v1.5 test (Roche Molecular Systems)ではLCx HIV RNA Quantitative assay (Abbott Laboratories)に比べ有意に低値を示した。プライマーとプローブ結合部位でのヌクレオチドの不整合が、定量差の原因である。HIV-1の遺伝的多様性がアッセイにおける検出と定量に影響を与えることに注意すべきである。	
60060	2006/06/20	HTLV	International Conference on Emerging Infectious Diseases 2006; Mar 19-22;	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名の血漿検体を用いて、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。	4
60056	2006/06/02	インフルエンザ	AABB Weekly Report 2006; 12(2): 1-3	2006年1月5～6日に米国保健省血液安全安定供給諮問委員会で、インフルエンザの大流行とその血液供給に及ぼす影響について議論された。特に短期生存型血小板の供給が脅かされることが強調された。また血液供給者の潜在的ウイルス感染問題も含めて、安全な血液供給に関する研究がさらに必要であるとされ、保健省が取り組むべき対応策を可決した。	
60056	2006/06/02	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。	5
60060	2006/06/20	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。	6
60056	2006/06/02	ウイルス感染	J Clin Microbiol 2005; 43: 5428-5434	米国で1987年から1996年の間にHIV感染小児患者57例から採取し、凍結保存した末梢血単核細胞(PBMC)と2002年から2003年に健常者19例から採取した新鮮PBMCにおいてヒトパピローマウイルス(HPV) DNAを調べた。患者8例と健常者3例がHPV型16ゲノムの2つのサブグループの大部分に陽性であり、これら11のPBMC検体すべてで検出されたHPVゲノムはエピソーム型として存在した。PBMCはHPVのキャリアであり、血液を介してHPVを広めるおそれがあることが示唆された。	
60062	2006/06/20	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。	7
60059	2006/06/20	ウイルス感染	J Med Virol 2006; 78: 693-701	ヒトにおけるVesivirus感染を調べるために、米国オレゴン州の赤十字血液検査研究所で1996年から1999年に供血者から集められた血清765例について検査した。その結果、抗体陽性率は、健常ドナー群で12%(374例中44例）、高ALT値群で21%(350例中73例）、感染が原因と疑われる肝炎患者群で29%(41例中12例）、輸血または透析に関連した肝炎患者群で47%(15例中7例）であった。さらに、RT-PCR試験を実施した112血清検体の内11検体（9.8%）が陽性であり、既知のVesivirusと関係があった。これらのデータは、検査された血液ドナーの中に、過去にVesivirusに感染したヒトも、またVesivirusウイルス血症者もいることを示している。	

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60056	2006/06/02	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。	
60062	2006/06/20	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。	
60102	2006/07/31	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。カナダ公衆衛生局は旅行者に対し、蚊に刺されないように等、注意を呼びかけている。	8
60080	2006/07/19	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。	9
60056	2006/06/02	ウエストナイルウイルス	CDC http://www.cdc.gov/ncidod/dvbid/westnile/qa/transfusion.htm	2005年8月-9月にﾆｭｰﾖｰｸとﾍﾟﾝｼﾙﾊﾞﾆｱで臓器移植を受けたレシピエントがウエストナイルウイルス感染した件に関連するQ＆A。臓器移植による感染は、ドナーの血液が核酸増幅試験陰性、IgM、IgG抗体陽性の場合にも起こる可能性がある。	
60056	2006/06/02	ウエストナイルウイルス	CDC/MMWR 2005; 54(Dispatch): 1-3	2005年9月、米国で共通のドナーから臓器移植を受けたレシピエント4名中3名にWNV感染が確認された。ドナーから採取された血清および血漿サンプルの検査でWNV-IgM抗体、IgG抗体は陽性を示したが、WNV-RNAは陰性であった。	
60056	2006/06/02	ウエストナイルウイルス	朝日新聞 2005年10月3日	厚生労働省は2005年10月3日、米国から日本帰国した男性会社員が米国で流行中のウエストナイル熱と診断されたと発表した。国内初の感染例である。	
60056	2006/06/02	エボラ出血	Nature 2005; 438: 575-576	ガボンおよびコンゴで2001年から2003年にかけて発生したヒトと大型霊長類におけるエボラ流行時に採集された1030の小型脊椎動物において、エボラウイルスについて調べた。エボラウイルスに特異的な抗体が3種類のコウモリの血清中で検出された。エボラウイルスのヌクレオチド配列が同じ種類のコウモリの肝臓と脾臓で検出されたが、腎臓、心臓、肺からは検出されなかった。また他の動物からは検出されなかった。驚くべきことに、抗体陽性の動物はすべてPCR陰性であり、PCR陽性の動物はすべて抗体陰性であった。これはPCR陽性の動物は感染から日が浅く、免疫反応が検出できる前に検査されたためと思われる。	

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60062	2006/06/20	クロストリジウム感染	N Engl J Med 2005; 353: 2433-2441	米国において、毒性、抗菌薬耐性、あるいはその両方が高まったClostridium difficileの新菌株の出現により、関連疾患の発生率と重症度が上昇している可能性が示唆されている。2000年から2003年に本菌関連疾患の集団発生が起きた8医療施設から得た本菌の分離株187株を、2001年以前の分離株データベースと比較した。その結果、全施設の分離株で同定された最近のBI/NAP1株は、ガチフロキサシンとモキシフロキサシンに耐性を示すことが明らかとなった。	
60056	2006/06/02	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。	
60056	2006/06/02	コロナウイルス感染	Science 2005; 310: 676-679	2004年3月から12月に、中国の4地区から408匹のコウモリを集め、血液、糞、唾液を採取し、血清検体および糞または唾液由来cDNAを、各々独立に、異なった方法で、二重盲検により分析した。その結果、ある種のコウモリが重症急性呼吸器症候群(SARS)の病原体であるSARSコロナウイルス(SARS-CoV)に非常に近いコロナウイルスの自然宿主であることが明らかになった。これらのウイルスはSARS様コロナウイルス(SL-CoV)と名づけられ、ヒトやジャコウネコから分離されたSARS-CoVより遺伝的多様性が高い。ヒトやジャコウネコから分離されたSARS-CoVは系統発生学的にSL-CoVの範疇に入り、SARS発生の原因ウイルスがSL-CoV群の一員であったことを示す。	
60056	2006/06/02	デング熱	Blood 2005; 106: Abstract #5331	骨髄移植後の最初の再発時に敗血症と不可逆性ショックを発症し、死後解剖でデングウイルス4型感染が判明した急性リンパ性白血病(ALL)小児患者について報告する。1994年11月にプエルトリコで兄弟からの骨髄移植を受けた6歳の少女は移植後5日目に全身紅斑、6日目に発熱を発症し、抗生物質の投与にかかわらず、不可逆的ショックを起こし、11日目に死亡した。死後解剖で血液、腹水、肝臓、脾臓からデングウイルス4型が検出され、PCRで確認された。ドナーの血液をさらに検査したところ、デングウイルス4型のIgM抗体が検出され、患者ウイルスの培養は、ドナーの急性力価と一致した。デングウイルス感染は流行地域で輸血や骨髄移植を受けた患者の死亡原因となりうる。	
60056	2006/06/02	デング熱	ProMed20050928-0040	2005年9月、ベネズエラ、シンガポール、カリブ海マルチニーク島、マレーシアでデング熱が流行している。死亡者も多数でている。	
60069	2006/06/29	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。発育鶏卵培養によりウイルスを分離し、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(HA)とノイラミニダーゼ(NA)遺伝子について系統遺伝学的分析を行ったところ、HAは2004N5N1とは異なり、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。NAはタイで分離された2004-2005H5N1と同じアミノ酸欠損を示した。本症例では血液中にウイルスが存在したことから、感染者の血液の取り扱いは注意深く行うべきである。	10
60056	2006/06/02	鳥インフルエンザ	Nature 2005; 437 : 1108	2005年2月、ベトナムのトリインフルエンザ感染者においてオセルタミビルに耐性を示すH5N1型ウイルスが発見された。患者は予防量から開始し、のち高用量（治療量）投与され、回復した。高用量投与後はウイルスは分離されなかった。フェレットに感染させた実験で、オセルタミビル耐性ウイルスはザナミビルには感受性を示した。	

血対ID	受理日	感染症（PT）	出典	概要	
60056	2006/06/02	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。	
60062	2006/06/20	鳥インフルエンザ	WHO/CSR 2005年11月17日	中国で初めて2例の高病原性トリインフルエンザウイルス（H5N1)感染症例が確認された。1例は回復したが、もう1例は死亡した。	
60056	2006/06/02	パルボウィルス	Clin Infect Dis 2005; 41: 1201-1203	パルボウイルスに急性感染後のウイルス動態の再評価により、症状が早期に消失したにもかかわらず、、本ウイルスは宿主から急速には除去されないことが示された。	
60058	2006/06/14	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。	
60085	2006/07/24	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。	
60062	2006/06/20	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のバリンがホモ接合体であった。今まで調べられたvCJD患者は、メチオニン/バリン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がメチオニンのホモ接合体サブグループであり、バリンホモ接合体サブグループがvCJDに対して感受性があることが初めて示された。この遺伝子型のvCJD感染者は長い潜伏期間を有している可能性があり、この間に水平感染が供血または無症候期の感染者に使用された汚染手術用具のいずれかから起きる可能性がある。	11
60085	2006/07/24	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。	
60056	2006/06/02	変異型クロイツフェルト・ヤコブ病	J Virol 2005; 79: 13794-13796	慢性消耗病(CWD)感染ミュールジカの脳組織を、リスザルの脳内に接種したところ、リスザルは進行性神経変性疾患を発現した。リスザルの脳組織にはPrPresが検出され、海綿状変性が認められた。霊長類にCWDが感染した初めての報告である。	
60056	2006/06/02	変異型クロイツフェルト・ヤコブ病	J Virol 2006; 80: 322-331	酸性ドデシル硫酸ナトリウム(SDS)によるプリオンの不活性化について検討した。ハムスターSc237プリオンおよびヒト散在性クロイツフェルト・ヤコブ病(sCJD)プリオンの酸性SDS暴露による不活性化には、SDS濃度、暴露期間、温度が関係した。ヒトsCJDプリオンはハムスターSc237プリオンに比べ、不活性化に10万倍以上抵抗性を示した。ステンレス鋼線に付着したヒトsCJDプリオンは酸性SDSとオートクレーブの併用で除去された。この知見は手術器具や歯科用機器などのプリオン不活性化に適したシステムの基礎となる。	

血対ID	受理日	感染症（PT）	出典	概要	
60056	2006/06/02	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。	12
60062	2006/06/20	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。BSEからヒトへの感染は種の壁の存在によって制限を受けているが、vCJDのヒトからヒトへの感染は実質的に壁が低くなっているように思われる。さらに、コドン129の遺伝型に関係なく、輸血のようなルートによって、vCJDの2次感染が起こりやすい可能性がある。潜伏期間の長い疾患はこれらのモデルによって予測ができ、疾患の伝播拡大の危険性や有意な公衆衛生上の問題を示すであろう。	13
60056	2006/06/02	変異型クロイツフェルト・ヤコブ病	Nat Med 2005; 11: 1137-1138	イタリアのサッサリ地方で飼育されている818頭のヒツジについて調べたところ、そのうち261頭がプリオン病に対する感受性を与えるPrnp対立形質を有していた。7頭が明らかなスクレイピーであったが、脳、リンパ節、扁桃腺でPrPScが検出された。スクレイピーのヒツジ全てと無作為に選んだ健康なヒツジ100頭について乳腺を組織学的に調べたところ、乳腺炎とスクレイピーを併発していた4頭では乳腺においてPrPScが検出された。30 km離れた別の群れのヒツジ272頭についても同様の調査を行ったところ、1頭が同様の所見を呈した。慢性的な炎症とスクレイピーの併発により、PrPScの沈着が予期せぬ組織に広がることが示された。	
60062	2006/06/20	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。	14
60056	2006/06/02	変異型クロイツフェルト・ヤコブ病	ProMED20060112-0070	英国保健省の月間統計によると2006年1月6日時点でCJD死亡患者総数（BSEと関連があると思われるvCJDを含む）は153例で、内訳はvCJD確定例における死亡患者109例、vCJD可能性例における死亡患者（神経病理学的に未確定）43例、vCJD可能性例における死亡患者（神経病理学的確定実施中）1例であった。存命中のvCJD患者は6例で、vCJD確定例および可能性例総数は159例で前月から変化はなかった。	
60062	2006/06/20	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。	

血対ID	受理日	感染症（PT）	出典	概要	
60056	2006/06/02	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。	15
60056	2006/06/02	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。	16
60085	2006/07/24	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。	17
60056	2006/06/02	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。この患者は、英国で供血後にvCJDを発症したドナーから輸血されたことが判明し生存しているおよそ30人の一人だった。対象者は全員vCJD暴露の可能性があることを通知され、手術などの医療措置を介したvCJD伝播の危険を減らすための予防措置を講じるよう求められている。	
60056	2006/06/02	マラリア	ProMed20051015-0070	ﾌﾗﾝｽ人旅行者1名が2005年8月～9月ﾄﾞﾐﾆｶ共和国東部を旅行した後に熱帯性マラリアを発症した。ﾏﾗﾘｱ薬の服用歴なし。過去12ヶ月以内に輸血、臓器移植歴もなし。	
60056	2006/06/02	レンサ球菌感染	Clin Microbiol Infect 2005; 11: 919-924	スペインの2つの大病院で行われた成人における肺炎連鎖球菌菌血症回顧試験で、1020件中108件(10.6 %)が病院内肺炎球菌血流感染(NPBI)と同定された。この内77例のデータが分析可能であったが、入院後、血液培養が陽性になるまでは3～135日（中央値17日）で、基礎疾患は悪性腫瘍(31 %)、慢性閉塞性肺疾患(28.6 %)、心不全(16.9 %)、慢性腎不全(15.6 %)、肝硬変(13 %)、HIV感染(13%)であった。患者の31.2 %が重度の敗血症、11.7 %が敗血症ショック、3.9 %が多臓器不全を呈した。原因菌の血清型のうち、78 %は23価多糖体ワクチンに含まれていた。35名(45.5 %)の患者が死亡し、そのうち21名(27.3 %)がNPBIに関連すると考えられた。	
60056	2006/06/02	感染（梅毒）	Eurosurveillance 2005; 10(11): 051110	1999年以来、スウェーデンでは梅毒症例数が増加している。男性と性交渉する男性の間で激増しているためで、2004年は前年比7%増の192例で、1980年代半ば以来最高となった。感染の60%は男性間性交渉、38%は男女間性交渉によるもので、約半数(97例)がストックホルム郡で報告されている。ストックホルム郡外の症例のうち2例は海外で血液製剤により感染した。	

血対ID	受理日	感染症（PT）	出典	概要	
60058	2006/06/14	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。	18
60062	2006/06/20	寄生虫感染	Transfusion 2005; 45: 1804-1810	コネチカット州のバベシア流行地及び非流行地の血液ドナーそれぞれ1745例の血清をBabesia microti抗体について調べた。流行地の血清学的陽性血液ドナーは24例(1.4%)で，非流行地の陽性血液ドナー(6例、0.3%)より多かった。また，血清学的陽性の血液ドナー19例のうち10例(53%)がPCRによりBabesia microtiに陽性であった。輸血により本寄生虫血症が伝播するおそれがある。	
60056	2006/06/02	細菌感染	Transfusion 2005; 45: 1845-1852	2004年3月にアメリカ赤十字の36の地域血液センターすべてにおいて、成分採血由来の血小板製剤における細菌汚染についてルーチンの品質管理試験を行った。細菌試験の最初の10ヶ月で350,658例中226例が初期陽性であった。初期陽性のものにつき再度検体採取したところ、68例で細菌汚染が確認され、陽性率は0.019 %であった。単離された細菌はブドウ球菌属(47.1 %)、連鎖球菌属(26.5 %)、グラム陰性菌(17.6 %)であった。スクリーニングで陰性であった成分に対して、敗血症性輸血反応と疑わしい症例が3例特定され、これらはすべてコアグラーゼ陰性ブドウ球菌が原因とされた。	
60056	2006/06/02	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。	
60056	2006/06/02	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。	19

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No. 32

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研究報告の概要

○日本固有のE型肝炎ウイルスの分子追跡

日本固有と思われるE型肝炎ウイルス(HEV)株の起源はおそらく外国由来であるが、いつどこから国内に流入したのかについては不明のままである。日本国内で回収されたHEV株で遺伝子型3の24株、遺伝子型4の24株が、821nt RNAポリメラーゼ遺伝子フラグメントから構成される系統樹において、外来株とは明らかに異なるクラスターを示した。進化速度は約0.8x10(-3)ヌクレオチド置換/領域/年で、HEVの個体群統計歴の追跡が可能であり、日本固有のHEVの起源は、数種のヨークシャー種のブタが英国から日本に輸入されたおよそ1900年頃であることが示唆された。興味深いことに、日本における遺伝子型3の進化成長は1920年代から緩徐であるのに、遺伝子型4は1980年代から急速に広がっている。結論すると、こうしたデータは、日本におけるHEVの土着化と蔓延は、豚肉の摂食の大衆化と関係していたことを示唆する。

報告企業の意見	今後の対応
日本固有のHEVの起源は、数種のヨークシャー種のブタが英国から日本に輸入されたおよそ1900年頃であり、日本におけるHEVの土着化と蔓延は、豚肉の摂食の大衆化と関係していたとの報告である。	日本赤十字社では、厚生労働省科学研究「本邦に於けるE型肝炎の診断・予防・疫学に関する研究班」と共同して、献血者におけるHEV感染の疫学調査を行っている。北海道における輸血HEV感染報告を受け、試験的に北海道では生肉の摂取の有無について問診の有用性を検討し研究的NATを行うなど安全対策を実施している。今後もHEV感染の実態に関する情報の収集及び安全対策に努める。

使用上の注意記載状況・その他参考事項等

解凍赤血球濃厚液「日赤」
照射解凍赤血球濃厚液「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

(一)

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Short Communication

Molecular tracing of Japan-indigenous hepatitis E viruses

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The ancestor(s) of apparently Japan-indigenous strains of *Hepatitis E virus* (HEV) was probably of foreign origin, but it remains unclear when and from where it made inroads. In this study, 24 genotype 3 and 24 genotype 4 HEV strains recovered in Japan each showed a significant cluster, clearly distinct from those of foreign strains, in the phylogenetic tree constructed from an 821 nt RNA polymerase gene fragment. The evolutionary rate, approximately $0{\cdot}8 \times 10^{-3}$ nucleotide substitutions per site per year, enabled tracing of the demographic history of HEV and suggested that the ancestors of Japan-indigenous HEV had made inroads around 1900, when several kinds of Yorkshire pig were imported from the UK to Japan. Interestingly, the evolutionary growth of genotype 3 in Japan has been slow since the 1920s, whereas genotype 4 has spread rapidly since the 1980s. In conclusion, these data suggest that the indigenization and spread of HEV in Japan were associated with the popularization of eating pork.

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Transmission of *Hepatitis E virus* (HEV) occurs primarily by the faecal–oral route through contaminated water supplies in developing countries (Purcell & Emerson, 2001). Additionally, increasing evidence has indicated that hepatitis E is a zoonosis (Harrison, 1999; Kabrane-Lazizi *et al.*, 1999; Meng *et al.*, 1997, 1998, 2002; Nishizawa *et al.*, 2003; Okamoto *et al.*, 2001; Tei *et al.*, 2003; Yazaki *et al.*, 2003). It has recently been suggested that zoonotic, food-borne transmission of HEV from domestic pigs, wild boars or wild deer to humans plays an important role in the occurrence of domestic infections of hepatitis E in Japan, where people have unique habits of ingesting raw fish (sushi or sashimi) and uncooked or undercooked meat (also organ meats, such as raw liver) (Matsuda *et al.*, 2003; Tamada *et al.*, 2004). Thus, it seems that HEV infection is now autochthonous in Japan. It remains unclear, however, when and from where the ancestral HEV strains made inroads and have spread in

The GenBank/EMBL/DDBJ accession numbers for the HEV nucleotide sequences reported in this paper are shown in Fig. 1.

Supplementary tables are available in JGV Online.

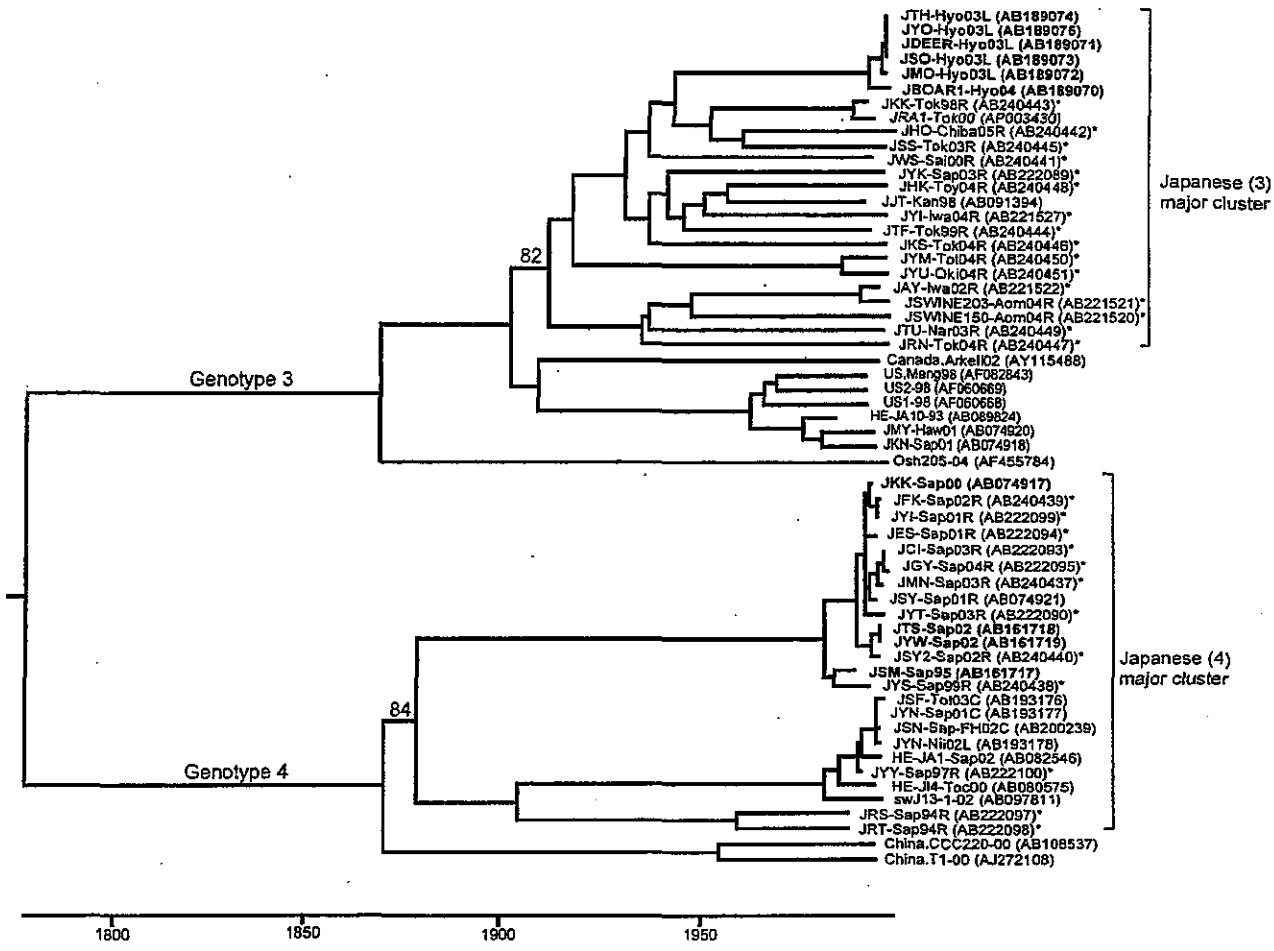

Fig. 1. Phylogenetic tree of the partial RNA polymerase region of the HEV genome. Twenty-four genotype 3 and 24 genotype 4 strains in Japan showed each significant cluster to have a high bootstrap value and to be distinct from other reference sequences (USA, Canada and Japanese minor strains in genotype 3; Chinese strains in genotype 4). Genetic distances have been transformed into a time scale of years by using estimates of the molecular clock ($0{\cdot}84 \times 10^{-3}$ nucleotide substitutions per site per year). Ten strains in bold are used for linear regression in Fig. 2. Strain names are followed by prefecture or city names in Japan: Hyo, Hyogo; Tok, Tokyo; Sai, Saitama; Sap, Sapporo; Iwa, Iwate; Kan, Kanagawa; Oki, Okinawa; Aom, Aomori; Nar, Nara; Tot, Tottori; Nii, Niigata; Toc, Tochigi; Toy, Toyama. Asterisks indicate strains that were newly sequenced in this study.

Japan. In this study, we first estimated the evolutionary rate of HEV by using Japan-indigenous genotype 3 and genotype 4 strains, which were phylogenetically distinct from the other strains in foreign countries. Then, based on this evolutionary rate, we traced the demographic history of HEV in Japan.

For linear-regression analyses within significant clusters, two independent datasets were applied: one was a Hyogo cluster (genotype 3) with JMO-Hyo03L, JTH-Hyo03L, JSO-Hyo03L, JYO-Hyo03L, JDEER-Hyo03L (these five isolates were obtained in April 2003) and JBOAR1-Hyo04 (April 2004) (Takahashi *et al.*, 2004a), and another was a Sapporo cluster (genotype 4) with JSM-Sap95 (March 1995), JKK-Sap00 (November 2000), JYWSap02 (August 2002) and JTS-Sap02 (September 2002) (Takahashi *et al.*, 2004b). GenBank accession numbers for these strains are given in Fig. 1. To elucidate the epidemiological history of the HEV population in Japan, 48 known and newly sequenced HEV strains ($n=24$ for each of genotype 3 and 4) were used for molecular-evolutionary analyses. The nucleotide sequences of 28 strains for the molecular-clock analyses were determined in this study (the other 20 sequences dealt with in this paper were available from GenBank).

Nucleic acids were extracted from serum samples (50 µl) by using a commercial Smitest EX-R & D kit (Genome Science) and precipitated in a 2 ml tube. The pellet was air-dried for 15 min and then suspended in 10 µl autoclaved distilled water containing 10 U RNase inhibitor ml^{-1} (TaKaRa

Shuzo). A sequence spanning 821 nt in the RNA-dependent RNA polymerase region (corresponding to nt 3961–4781 of the prototype Burmese HEV strain; GenBank accession no. M73218), including the GDD motif, was amplified by PCR in three overlapping regions with 20-mer primers deduced from known HEV sequences. Reverse transcription was performed at 50 °C for 60 min with the Thermo-Script RT system (Invitrogen), and the first- and second-round PCRs were carried out in the presence of Platinum *Taq* DNA Polymerase High Fidelity (Invitrogen). The final products were sequenced in an ABI 377 DNA sequencer (PE Biosystems) with an ABI Prism BigDye kit (Applied Biosystems). The sequences determined were utilized to confirm HEV genotypes and to construct phylogenetic trees. The reliability of the phylogenetic tree was assessed by bootstrap-resampling tests.

A reconstructed tree was built on the RNA polymerase region by using a heuristic maximum-likelihood (ML) topology search with stepwise addition and nearest neighbour-interchange algorithms. Tree likelihood scores were calculated by using the HKY85 model (Hasegawa *et al.*, 1985) with the molecular clock enforced, using PAUP version 4.0b8. Using the estimated topology, all possible root positions were evaluated under a single-rate dated-tips (SRDT) model with the computer software TipDate v1.2 and the root that yielded the highest likelihood was adopted (Rambaut, 2000). The program provided an ML estimate of the rate and also the associated date of the most recent common ancestor of the sequences, using a model that assumed a constant rate of nucleotide substitution. The molecular clock was tested by a likelihood-ratio test between the SRDT model and a general unconstrained branch-length model [different-rate (DR) model].

For estimates of demographic history, a non-parametric function $N(t)$, also known as a skyline plot, was obtained by transforming the coalescent intervals of an observed genealogy into a piecewise plot that represented an effective population size through time (Pybus *et al.*, 2001; Pybus & Rambaut, 2002). A parametric ML was estimated by several models with the computer software GENIE v3.5 to build a statistical framework for inferring the demographic history of a population on phylogenies reconstructed from sampled DNA sequences (Pybus & Rambaut, 2002). This model assumes a continuous epidemic process in which the viral transmission parameters remain constant through time. Model fitting was evaluated by likelihood-ratio tests of the parametric ML estimates (Lemey *et al.*, 2003; Pybus *et al.*, 2003; Tanaka *et al.*, 2005). Approximate 95 % confidence intervals for the parameters were estimated by using the likelihood-ratio test statistics.

A phylogenetic tree in the partial RNA polymerase region of the HEV genome is represented in Fig. 1. A functional gene, such as the RNA polymerase gene, is suitable for molecular-evolutionary analyses based on the neutral theory, because the substitution of functional genes is based on the neutral theory. The 24 genotype 3 and 24 genotype 4 strains in Japan showed a significant cluster with a high bootstrap value, which was the major Japanese cluster distinct from other strains found in foreign countries by molecular-evolutionary analyses. Such a significant cluster is suitable for the following coalescent analysis. Additionally, the tree topology based on the RNA polymerase region, including functional genes, was quite similar to that based on complete genomes (data not shown).

To determine the evolutionary rate of HEV, the 48 Japan-indigenous HEV strains (Fig. 1) were subjected to further molecular-evolutionary analyses. The molecular-evolutionary rate was estimated by two independent methods. In brief, linear-regression analyses using highly similar strains, i.e. six genotype 3 strains in Hyogo and four genotype 4 strains in Sapporo, indicated that a molecular-evolutionary rate was $(0{\cdot}81–0{\cdot}88) \times 10^{-3}$ nucleotide substitutions per site per year (Fig. 2). Second, TipDate (v1.2) was used to compare the DR model with the single-rate (SR) and SRDT models. The SRDT model provided an adequate fit to the data ($P > 0{\cdot}05$; see Supplementary Table S1, available in JGV Online). Based on the SRDT model, the mean rate of nucleotide substitutions was estimated to be $(0{\cdot}81–0{\cdot}94) \times 10^{-3}$ nucleotide

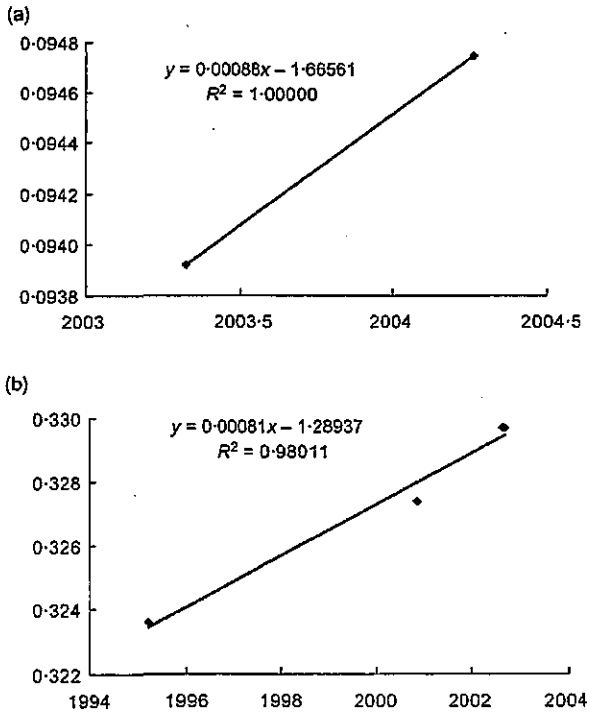

Fig. 2. Linear-regression analyses within the partial RNA polymerase region for evolutionary rate of HEV. (a) The evolutionary rate of genotype 3 in the Hyogo cluster is estimated to be $0{\cdot}88 \times 10^{-3}$ nucleotide substitutions per site per year; (b) the evolutionary rate of genotype 4 in the Sapporo cluster is estimated to be $0{\cdot}81 \times 10^{-3}$ nucleotide substitutions per site per year.

substitutions per site per year, which was similar to the rate for *Hepatitis C virus* (Ina *et al.*, 1994; Tanaka *et al.*, 2002). When we used $0{\cdot}84 \times 10^{-3}$ nucleotide substitutions per site per year, which was based on all 48 sequences (24 genotype 3 and 24 genotype 4), the time of the most recent common ancestor of Japan-indigenous genotype 3 was estimated to be in the 1900s (95 % confidence interval, 1902–1917) and that of genotype 4 was approximately in the 1880s (1881–1898) (Fig. 1).

Based on the phylogenetic tree, the effective number of HEV infections through time, $N(t)$, was analysed by using a skyline plot for the Japan-indigenous HEV strains. The parameters for several models in GENIE v3.5 were examined (see Supplementary Table S2, available in JGV Online). Time t was then transformed to year by using the constant rate ($0{\cdot}84 \times 10^{-3}$ nucleotide substitutions per site per year), assuming the collecting time to be the present. Fig. 3 shows the skyline plots and population growth for the HEV strains, according to a specific demographic model in GENIE v3.5 with three parameters and a piecewise-expansion growth model, which was evaluated by likelihood-ratio testing (Ina *et al.*, 1994; Lemey *et al.*, 2003; Pybus *et al.*, 2003; Tanaka *et al.*, 2005). Our estimates of the effective numbers of HEV infections showed a transition from constant size to exponential growth in the 1920s (95 % confidence interval, 1916–1930) among the genotype 3 population (Fig. 3a), whereas the rapid exponential growth among the genotype 4 population was dated in the 1980s (1978–1990) (Fig. 3b).

Because the natural course of HEV infection in human beings and animals is usually transient, not persistent as in the cases of hepatitis B and C viruses, it is almost impossible to estimate the molecular-evolutionary rate of HEV by using serial samples from an individual host. However, even though HEV does not persist in individual hosts, it could persist in the community by hopping from host to host successively. The first study attempting to estimate the number of synonymous mutations per synonymous site (k_s) of *Hepatitis A virus* (HAV) was reported by Sánchez *et al.* (2003). The estimated k_s values from HAV strains isolated from a clam-associated outbreak varied from 0·038 for VP0 to 0·29 for VP1. Similarly, we estimated the evolutionary rate of HEV by using Japan-indigenous genotype 3 and genotype 4 strains isolated over time. The rate was estimated to be approximately $0{\cdot}8 \times 10^{-3}$ nucleotide substitutions per site per year by two independent methods, which was around half of our previously estimated rate (Takahashi *et al.*, 2004b). One of the reasons is that the molecular-evolutionary rate would depend on estimated genes; the previous report (Takahashi *et al.*, 2004b) used complete sequences, whereas this study used only RNA polymerase sequences. Another reason is that the previous extrapolation of substitution rate on pairwise (direct) comparisons can give overestimates of the molecular clock and hence divergent times of HEV species, as reported previously (Ina *et al.*, 1994). Based on the molecular clock, we traced the demographic history of HEV in Japan and the indigenization time was suggested to be similar (approx. 1900), but the spread time was quite different, between HEV genotypes 3 and 4 (1920s versus 1980s). Interestingly, in addition, the evolutionary growth of genotype 3 has been quite slow since the 1920s, whereas genotype 4 strains have spread rapidly in Sapporo since the 1980s.

Zoonosis has been implicated in HEV transmission. The first animal strain of HEV to be isolated and characterized was a swine HEV from a pig in the USA in 1997 (Meng *et al.*, 1997). Since then, many swine HEV strains, which exhibit extensive genetic heterogeneity, have been identified worldwide and shown to be genetically related closely to strains of human HEV (Chandler *et al.*, 1999; Hsieh *et al.*, 1999; Huang *et al.*, 2002; Okamoto *et al.*, 2001; Wang *et al.*, 2002). Recent findings suggested an interspecies HEV transmission between boar and deer in their wild life (Takahashi *et al.*, 2004a) and that both animals might serve as an infection source for human beings. More recently, wild mongoose was newly added to the list of HEV-reservoir animals in Japan

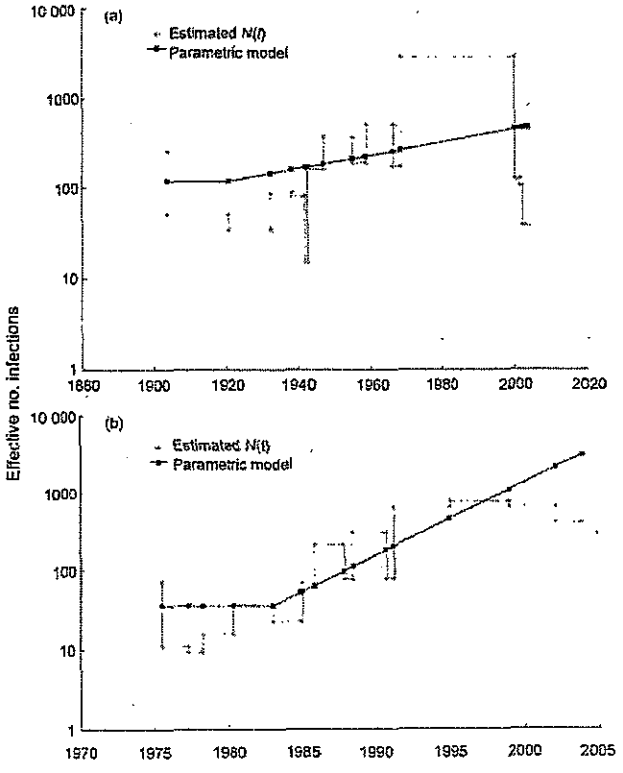

Fig. 3. ML estimates of $N(t)$ on the effective number of (a) HEV genotype 3 and (b) HEV genotype 4 infections in Japan. The parametric model is indicated by the black line and stepwise plots by the grey line, which represents corresponding non-parametric estimates of $N(t)$ (number as a function of time). Genetic distances have been transformed into a time scale of years by using estimates of the molecular clock in the partial RNA polymerase region of HEV.

(Nakamura *et al.*, 2006). Notwithstanding the importance of these wild animals, pigs for food must be the major reservoirs of HEV: a recent Japanese study indicated that anti-HEV antibodies were detected in 1448 (58 %) of 2500 pigs from 2 to 6 months of age at 25 commercial swine farms in Japan (Takahashi *et al.*, 2003). The importance of transmission of HEV from pigs to humans was further supported by a recent field study in Indonesia: Muslim people, for whom it is a taboo to eat or contact pigs, were significantly less frequently positive for anti-HEV than Hindu people (2·0 vs 20 %) (Surya *et al.*, 2005).

Our molecular-evolutionary analyses suggested that HEV entered Japan around 1900. If we have traced the origin of Japan-indigenous HEV correctly back to about 100 years ago, what happened at that time in relevance to HEV's indigenization? Several kinds of Yorkshire pig were imported for the first time in the history of Japan from the UK in 1900, by the Japanese government's policy to introduce excellent domestic animals for food in Western countries to Japan, as a measure to nutritionally strengthen the people (especially soldiers) of this formerly vegetarian country. Since then, the Yorkshire pigs have been propagated in Japan and, in the 1930s, thousands of pigs were reported all over Japan (http://okayama.lin.go.jp/history/2-3-1-2.htm), suggesting that the domestic spread of HEV might have been associated with the popularization of pigs for food in Japan. Indeed, a previous phylogenetic analysis of a 304 bp nucleotide sequence (ORF2) obtained from the two UK swine strains showed a close relationship with Japanese swine strains in genotype 3 (Banks *et al.*, 2004), indicating that Japanese genotype 3 may have been imported from the UK. On the other hand, Japanese genotype 4 strains were related phylogenetically to Asian strains in Taiwan and China. As the HEV found in wild boars living in the Iriomote Island, near Taiwan, was of genotype 4 (unpublished results), the source of Japanese genotype 4 might be from Taiwan or the mainland of China. Note that a phylogenetic analysis showed that the Japanese swine and human HEV strains segregated into four clusters [three genotype 3 clusters (one major Japanese and two minor clusters) and one genotype 4 cluster], with the highest nucleotide identity being 94·4–100 % between swine and human strains in each cluster (Takahashi *et al.*, 2003), suggesting that swine have served as one of the most important reservoirs for HEV to be transmitted to humans. The possible risk factor for transmission of HEV was to have eaten uncooked or undercooked pig liver and/or intestine 1–2 months before the onset of hepatitis E in Hokkaido, Japan (Mizuo *et al.*, 2005). Such eating habits, which are particularly unique to those living in Hokkaido (Sapporo is one of the big cities there) in recent decades, might be one of the reasons that HEV has been widespread in this area since 1990, as supported by our molecular-evolutionary analyses in this study.

In conclusion, based on our present data, the indigenization and domestic spread of HEV in Japan are proposed to have been associated with the importation and popularization of pigs for food in Japan. However, there still remains a possibility of different scenarios. Another animal(s) might have carried the virus to Japan: for example, mongoose was imported from India to Japan in 1910 (Nakamura *et al.*, 2006).

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Contributions of authors are as follows: Y. T. performed molecular-clock analyses and wrote the manuscript; K. T. amplified and sequenced viral isolates; E. O. helped Y. T. with the molecular-clock analyses; Y. K., J.-H. K., K. S., A. M., A. H., H. M., H. S., Y. A. and T. K. provided HEV RNA-positive sera to K. T. for sequence determinations; M. M. supervised the molecular-clock analyses; and S. M. designed the study and helped Y. T. to write the manuscript. This work was supported in part by grants from the Ministry of Health, Labour and Welfare of Japan (200400676A) and from the United States–Japan Collaborative Medical Science Program (Hepatitis Panel). We greatly appreciate Dr Oliver G. Pybus (Department of Zoology, University of Oxford, Oxford, UK) for his enlightening advice on molecular-evolutionary analyses using GENIE v3.5.

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別紙様式第2-
番号 14

医薬品
医薬部外品　　研究報告　調査報告書
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識別番号・報告回数			報告日	第一報入手日 2006年5月8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	人ハプトグロビン		研究報告の公表状況	肝臓 2006; 47 (Supplement 1):A168	公表国 日本	
販売名 (企業名)	ハプトグロビン注－ヨシトミ(ベネシス)					

研究報告の概要

＜目的＞
E型肝炎ウイルス(HEV)は主として経口感染するが、最近HEVの血液感染が問題となっている。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抗体の陽性頻度を検討した。
【対象と方法】
名古屋大学病院に通院中の血友病患者80例(男性79例:女性1例、血友病A61例:血友病B19例、年齢39.0±14.4歳)について検討した。HEV抗体はLiらの方法(JMedVirol2000;62:327-33)にて測定した。また全例においてA型肝炎ウイルス(HAV)抗体・HBs抗原・HBs抗体・HBc抗体・HCV RNA・HIV RNAを、48例においてGBV-CRNAを測定し、これらのウイルス/抗体の陽性率をHEV抗体陽性例と陰性例とで比較した。
＜結果＞
HEV抗体は13例(16.3%)で陽性であった。患者年齢はHEV抗体陽性例で46.9±17.9歳、HEV抗体陰性例で37.4±13.1歳であり、陽性例の方が高かった(p=0.0346)。HEV抗体陽性例/陰性例とで他のウイルスの状況を比較すると、HAV抗体陽性例は6例(46.2%)／22.4%、HBsAg陽性例は1例(7.7%)/0、HBsAb陽性例は8例(61.5%)/40例(59.7%)、HBcAb陽性例は10例(76.9%)/52例(77.6%)、HCV RNA陽性例は13例(100%)/63例(94.2%)、HIV RNAは5例(38.5%)/27例(40.3%)、GBV-C RNAは4例(44.4%)/12例(30.8%)であり、これらはいずれもHEV抗体陽性例と陰性例との間で差を認めなかった。
＜考察＞
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%、透析患者では9.4%と報告されている。今回の検討ではこれらのグループよりも血液感染のリスクが高いと考えられる血友病患者において16.3%とより高い陽性率が認められ、血友病患者におけるHEVの血液感染の可能性が示唆された。一方、HEV抗体陽性例と陰性例他のウイルスマーカーの陽性率には差は見られなかったが、HEV抗体陽性例で年齢が高く、血液製剤によるHEV伝播の時期を示唆する可能性が考えられた。

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献血者の血液については、HBs抗原、抗HCV抗体、抗HIV-1抗体、抗HIV-2抗体、抗HTLV-Ⅰ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人ハプトグロビンを濃縮・精製した製剤であり、ウイルス不活化・除去を目的として、製造工程において60℃、10時間の液状加熱処理及び濾過膜処理(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
供血者に比べて、血友病患者のHEV抗体陽性率が高く、血友病患者におけるHEVの血液感染の可能性が示唆されたとする報告である。従来、血友病患者におけるHEV感染状況については、弊社の知る限りにおいて、イタリアの血友病患者60人において抗HEV抗体陽性率はゼロであったとする報告（Lancet,1994,343,597-598）のみであった。本報告は、血漿分画製剤からのHEV伝播を示唆する初めての報告と思われ、今後同様の情報に注視する必要があると考える。	今後、同様の情報入手に努める。

(2)

P-9　血友病患者におけるE型肝炎ウイルス抗体の頻度

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森井正哉[1]，本多　隆[1]，林　和彦[1]，石上雅敏[1]，
中野　功[1]，豊田秀徳[2]，熊田　卓[2]，高松純樹[3]，
後藤秀実[1]
名古屋大学付属病院消化器内科[1]，大垣市民病院消化器科[2]，名古屋大学付属病院輸血部[3]

【目的】E型肝炎ウイルス（HEV）は主として経口感染するが，最近HEVの血液感染が問題となっている．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抗体の陽性頻度を検討した．
【対象と方法】名古屋大学病院に通院中の血友病患者80例（男性79例：女性1例，血友病A61例：血友病B19例，年齢39.0±14.4歳）について検討した．HEV抗体はLiらの方法（J Med Virol 2000；62：327-33）にて測定した．また全例においてA型肝炎ウイルス（HAV）抗体・HBs抗原・HBs抗体・HBc抗体・HCV RNA・HIV RNAを，48例においてGBV-C RNAを測定し，これらのウイルス/抗体の陽性率をHEV抗体陽性例と陰性例とで比較した．
【結果】HEV抗体は13例（16.3%）で陽性であった．患者年齢はHEV抗体陽性例で46.9±17.9歳，HEV抗体陰性例で37.4±13.1歳であり，陽性例の方が高かった（p＝0.0346）．HEV抗体陽性例/陰性例とで他のウイルスの状況を比較すると，HAV抗体陽性例は6例(46.2%)/22.4%，HBsAg陽性例は1例（7.7%）/0，HBsAb陽性例は8例（61.5%）/40例（59.7%），HBcAb陽性例は10例(76.9%)/52例(77.6%)，HCV RNA陽性例は13例(100%)/63例(94.2%)，HIV RNAは5例(38.5%)/27例(40.3%)，GBV-C RNAは4例（44.4%）/12例（30.8%）であり，これらはいずれもHEV抗体陽性例と陰性例との間で差を認めなかった．
【考察】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%，透析患者では9.4%と報告されている．今回の検討ではこれらのグループよりも血液感染のリスクが高いと考えられる血友病患者において16.3%とより高い陽性率が認められ，血友病患者におけるHEVの血液感染の可能性が示唆された．一方，HEV抗体陽性例と陰性例他のウイルスマーカーの陽性率には差は見られなかったが，HEV抗体陽性例で年齢が高く，血液製剤によるHEV伝播の時期を示唆する可能性が考えられた．

P-10　わが国の劇症肝炎，LOHFにおけるHEV感染の現状

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中山伸朗[1]，名越澄子[1]，三代俊治[2]，藤原研司[3]，
持田　智[1]
埼玉医科大学消化器内科・肝臓内科[1]，東芝病院研究部[2]，横浜労災病院[3]

【目的】国内固有のHEV株による急性肝疾患が注目されている．厚生労働省「難治性の肝疾患に関する研究」班が実施している劇症肝炎の全国集計でも，2001年の発症例ではE型症例が登録された．今後，HEV-RNAの測定が普及すると，成因不明例の中からE型症例が抽出される可能性がある．そこで，全国集計に登録された症例を対象に，血清HEV-RNAの測定に関する実態を明らかにする目的でアンケート調査を実施し，未測定と回答のあった症例では保存血清を回収してHEV-RNAを測定した．
【方法と成績】(1) 劇症肝炎，LOHFでウイルス感染が明らかでない症例の比率は，2001年50%（54/108），2002年54%（69/127），2003年50%（47/94）であった．これら症例の中で，血清HEV-RNAが測定されていたのは，2001年2%(1/54)，2002年17%(12/69)，2003年28%（13/47）であり，陽性であったのは2001年1例，2002年2例の計3例で，何れも北海道からの登録例であった．IgM-HEVの測定も含めると，2003年はウイルス感染の明らかでない症例の40%(19/47)でHEVに関する検索が実施されていたが，陽性例は認められなかった．(2) 保存血清は2001-2002年の症例は計47例（38%），2003年　29例（62%）で回収された．これらのうち，HEV-RNAは2001年に北海道から登録された劇症肝炎1例で陽性であった．単離したHEV株はgenotype IVで，全長塩基配列を解析したところ，約6ヶ月前に北海道で発症した急性肝炎重症型症例から検出された株と99.6%の相同性が認められた．
【考案と結語】劇症肝炎，LOHFの成因診断では，HEVに関する検索が普及しつつあるが，E型症例は北海道を除くと稀と考えられる．また，保存血清から単離されたHEV株は劇症化に関わる株である可能性があり，その特徴について更なる検討が必要である．

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医薬品
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識別番号・報告回数			報告日	第一報入手日 2006 年 5 月 15 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	人ハプトグロビン		研究報告の公表状況	日本輸血学会誌 2006;52(2):231	公表国 日本	
販売名 (企業名)	ハプトグロビン注ーヨシトミ(ベネシス)					

研究報告の概要

＜目的＞
北海道地区は HEV 陽性率が高く、輸血感染例も 2 例発生しており、HEV 浸淫地区と考えられている。そこで、同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。
＜対象＞
問診対象者：2004 年 11 月～2005 年 9 月に道内で献血した全献血者約 27 万人
スクリーニング対象者：2005 年 1～9 月に道内で献血した全献血者約 22 万人
＜方法＞
問診は、献血時に「3 ヶ月以内に豚、鹿、猪、あるいは動物種が不明の生肉、生レバーを食べたか」の HEV 問診を実施し、該当献血者に HEV NAT を実施した。
HEV NAT スクリーニングは、現行スクリーニング NAT 使用済み検体（20 本プール）を用いて行い、HEV NAT 陽性例については続いて HEV 抗体測定、HEV RNA 定量、遺伝子解析を行った。また、感染経路を調査する目的で喫食歴に関するアンケート調査を行った。
＜結果＞
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（男性 13 名、女性 7 名、HEV 問診該当者 1 名を含む）で、陽性率は 1/11,090(男性 1/10,180、女性 1/12,778)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。
陽性血から分離された HEV の遺伝型は 3 型 19 例、4 型 1 例であった。HEV 問診に該当しなかった陽性者 19 例に再度問い合わせたところ、14 例中 13 例が献血前に動物内臓肉を食していた。陽性血 4 例は結果判明前に血小板製剤が輸血に使用され、1 名は輸血感染が成立して E 型肝炎を発症し、1 名は未感染、2 名は感染未確認のまま原疾患で死亡した。
＜考察＞
北海道内の献血者の HEV RNA 陽性率は予想以上に高く喫食歴問診や抗体スクリーニングは HEV RNA 陽性者の排除には有効ではないと考えられたことから、HEV NAT スクリーニングによる検査体制の構築が必要と考えられる。

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) 本剤の原材料となる献血者の血液については、HBs 抗原、抗 HCV 抗体、抗 HIV-1 抗体、抗 HIV-2 抗体、抗 HTLV-Ⅰ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人ハプトグロビンを濃縮・精製した製剤であり、ウイルス不活化・除去を目的として、製造工程において 60℃、10 時間の液状加熱処理及び濾過膜処理(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
北海道内の献血者のHEV RNA陽性率は予想以上に高く、喫食歴問診や抗体スクリーニングはHEV RNA陽性者の排除には有効ではないと考えられたことから、HEV NATスクリーニングによる検査体制の構築が必要と考えられるとの報告である。 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。

③

テタノブリン

O-9　Multiple hit model を用いた生体内血小板寿命の測定

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【はじめに】血小板製剤の機能評価は，*in vitro* の状態だけでなく，生体内で血小板がどの程度生存するかという *in vivo* 試験が欠かせない．そのためには，血小板を放射性同位元素で標識したあと生体へ返血し，その生体内での消失度を評価する必要がある．今回はその第一ステップとして AuBuchon らが用いている（Transfusion，2005，45，1143—1150）multiple hit model による血小板機能の評価をおこなった．
【方法】健常人ボランティア男性2名において，全血採血によって新鮮血小板を分離した．この血小板に ^{111}In オキシン液にて，室温で20分間 incubate することによって ^{111}In をラベリングした後，20μCi を被験者へ直ちに返血した．採血後1から7日までの毎日と，10日目に採血し，^{111}I の放射活性を測定した．得られたデータを COST とよばれるプログラムへ入力し，multiple hit mode で血小板の%recovery と survival time を計算した．
【結果】得られた%Recovery はそれぞれ61，91% であった．また survival time は125，179hour であった．
【まとめ】今回，multiple hit model による *in vivo* での血小板機能評価を行うことができた．今後，この手法を用いて，長期保存血小板の機能評価を行う．

O-10　北海道地区における試行的 HEV プール NAT スクリーニングの実施

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松林圭二[1]，坂田秀勝[1]，徳島恵里奈[1]，田中聖子[1]，佐藤進一郎[1]，加藤俊明[1]，池田久實[1]，金光公浩[2]
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【目的】北海道は HEV 陽性率が高く，輸血感染例も2例発生しており，HEV 浸淫地区と考えられている．そこで，同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．
【対象】問診対象者は2004年11月～2005年9月に道内で献血した全献血者（約27万名）で，スクリーニング対象者は2005年1月～9月に道内で献血した全献血者（約22万名）である．
【方法】問診は献血時に「3ヶ月以内に豚，鹿，猪，あるいは動物種が不明の生肉，生レバーを食べたか」（HEV 問診）を質問し，該当献血者については HEV NAT を実施した．HEV NAT スクリーニングは，現行スクリーニング NAT 使用済み検体（20本プール）を用いて，Qiagen BioRobot 9604 で核酸抽出を行い，リアルタイム RT-PCR 法により検査した．HEV RNA 陽性例については HEV 抗体測定，HEV RNA 定量，遺伝子解析を行った．また，感染経路を調査する目的で喫食歴に関するアンケート調査を行った．
【結果】HEV 問診には765名（0.3%）が該当し，そのうち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（男性13名，女性7名，HEV 問診該当1名も含む）で，陽性率は1/11,090（男性1/10,180，女性1/12,778）となった．陽性者の多くは ALT 値が正常で HEV 抗体は陰性であった．陽性血から分離された HEV の遺伝子型は3型19例，4型1例であった．HEV 問診に該当しなかった陽性者19例に再度問い合わせたところ，14名中13名が献血前に動物内臓肉を食していた．陽性血4例は結果判明前に血小板製剤が輸血に使用され，1名は輸血感染が成立して E 型肝炎を発症し，1名は未感染，2名は感染未確認のまま原疾患で死亡した．
【考察】北海道内における献血者の HEV RNA 陽性率は予想以上に高く，喫食歴問診や抗体スクリーニングは HEV RNA 陽性者の排除に有効ではないと考えられたことから，HEV NAT スクリーニングによる検査体制の構築が必要と考えられる．

別紙様式第2-1

No. 28

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. 4. 26	該当なし	
一般的名称	人全血液		研究報告の公表状況	W. M. Switze, N. D. Wolfe, J. K. Carr, A. D. Garcia, V. Shanmugam, U. Tamoufe, J. Torimiro, A. T. Prosser, M. LeBreton, E. Mpoudi-Ngole, F. E. McCutchan, D. L. Birx, T. M. Folks, D. S. Burke, W. Heneine. International Conference on Emerging Infectious Diseases; 2006 Mar 19-22; Atlanta, Georgia.	公表国 米国	
販売名(企業名)	人全血液CPD「日赤」(日本赤十字社) 照射人全血液CPD「日赤」(日本赤十字社)					

研究報告の概要

○中央アフリカの猟師における新たなヒトTリンパ球向性ウイルス(HTLV)の出現

背景:HTLV-1の多様性は、サルTリンパ球向性ウイルス(STLV)-1の多系交雑種の伝播から生じたと思われる。しかし、ヒトと、既感染のヒト以外の霊長類(NHP)との接触が新たなHTLVの発生に寄与しているかどうかについてはほとんど判っていない。

方法:狩猟、屠殺、霊長類ペットの飼育などで、NHPの血液との接触が報告されている中央アフリカ人930例におけるHTLVの多様性を調査した。血漿検体はEIA測定法およびウエスタンブロット測定法を用いて血清学的に検査を行った。複数のウイルス領域の配列を、WB陽性例のPBL DNAからPCR増幅し、その後、既知の霊長類Tリンパ球向性ウイルス(PTLV)による系統発生解析を行った。

結果:プロウイルスの配列はWB陽性13例からPCRで増幅し決定した。系統発生解析によって猟師2名が新ウイルスに感染していることが明らかになり、このウイルスはHTLV-3およびHTLV-4と名づけられた。HTLV-3はSTLV-3と同属で、これまでヒトにおいては検出されていなかった。HTLV-4は既知のHTLV/STLVとは異なっており、遺伝的に等距離で、新たな系統を形成するものだった。11名は、これまでヒトにおいて認められていなかったマンドリルからのSTLV-1(n=3)および他のSTLV-1変異株(n=2)を始めとするHTLV-1と同属の様々なウイルスに感染していた。

結論:この個体群に2つの新しいHTLVが特定され、これまで知られていたものよりも幅広いHTLVの多様性が示される。こうした知見もまた、NHPへの曝露がHTLV出現に関与することを示唆するものである。

報告企業の意見	今後の対応
既感染のヒト以外の霊長類の血液と接触のあった中央アフリカ地域の人において、幅広い多様性をもつ新たな遺伝系統のHTLVが特定さ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

人全血液CPD「日赤」
照射人全血液CPD「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

④

coral trout and trevally (5 outbreaks each). There were 6 outbreaks of oily stools (keriorrhoea) from eating Escolar fish.

Conclusions: Seafood is responsible for approximately 1 in 5 identified foodborne outbreaks in Australia, although the median number of people affected is low. To prevent these outbreaks people should avoid eating certain fish or shellfish harvested from contaminated waters.

Slide Session 13

Epidemiology of Emerging Zoonotic Diseases I

Marquis I

Monday, March 20, 2006, 1:15 pm - 2:45 pm

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Emergence of Novel Human T-lymphotropic Viruses Among Central African Hunters

W. M. Switzer[1], N. D. Wolfe[2], J. K. Carr[3], A. D. Garcia[1], V. Shanmugam[1], U. Tamoufe[4], J. Torimiro[2], A. T. Prosser[1], M. LeBreton[2], E. Mpoudi-Ngole[4], F. E. McCutchan[3], D. L. Birx[1], T. M. Folks[1], D. S. Burke[2], W. Heneine[1];

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Background: HTLV-1 diversity appears to have resulted from multiple cross-species transmissions of STLV-1. However, little is known whether contact between humans and infected nonhuman primates (NHPs) continues to contribute to the emergence of novel HTLVs.

Methods: We investigated HTLV diversity among 930 central Africans reporting contact with NHP blood through hunting, butchering, and keeping primate pets. Plasma samples were tested serologically using EIA and WB assays. Sequences from several viral regions were PCR amplified from PBL DNA of WB reactive persons followed by phylogenetic analysis with known PTLVs.

Results: Proviral sequences were PCR-amplified from 13 WB reactive persons. Phylogenetic analysis revealed infection of two hunters with novel viruses we designated HTLV-3 and HTLV-4. HTLV-3 falls within the genetic diversity of STLV-3, a virus not seen before in humans. HTLV-4 is distinct and genetically equidistant from all known HTLVs/STLVs and formed of a new phylogenetic lineage. Eleven persons were also infected with a broad diversity of HTLV-1, including STLV-1 from mandrills (n=3) and other STLV-1 variants (n=2) not previously seen in humans.

Conclusions: We identify in this population two new HTLVs and demonstrate greater HTLV diversity than previously recognized. These findings also suggest that NHP exposure contributes to HTLV emergence.

(characters w/ spaces =1381; 199 words)

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From Civet Cats to Horseshoe Bats: Tracing the Origin of SARS

L. Wang[1], Z. Shi[2], S. Zhang[3], H. Field[4], P. Daszak[5], B. T. Eaton[1];

[1]CSIRO Australian Animal Health Laboratory, Geelong, AUSTRALIA, [2]Wuhan Institute of Virology, Chinese Academy of Sciences, Wuhan, CHINA, [3]Institute of Zoology, Chinese Academy of Sciences, Beijing, CHINA, [4]Department of Primary Industries and Fisheries, Brisbane, AUSTRALIA, [5]Consortium for Conservation Medicine, New York, NY.

Background: Severe acute respiratory syndrome (SARS) emerged in 2002-3 in southern China. Civets and other small mammals in the wildlife markets of Guangdong province were implicated in the transmission cycle. However, the true wildlife reservoir of the etiological agent of SARS, the SARS coronavirus (SARS-CoV), remained elusive. Prevention of future SARS outbreak will be difficult, if not impossible, to achieve without knowing the natural reservoir and the mechanism of spill over.

Methods: During March to December of 2004, 408 bats representing 9 species, 6 genera and 3 families, from four locations in China (Guangdong, Guangxi, Hubei and Tianjin) were sampled by trapping in their native habitat. Blood and fecal and throat swabs were collected; serum samples along with cDNA from fecal or throat samples were independently analysed, double-blind, using different methods by groups in China and Australia.

Results: Among six genera of bat species, three species from the genus *Rhinolophus* (horseshoe bats) demonstrated a high SARS-CoV antibody prevalence. The serological findings were corroborated by PCR analyses using primer pairs derived from the SARS-CoV. Three different SARS-like viruses (SL-CoV), were detected. Genome analysis demonstrated that SL-CoVs have an identical genome organization with that of SARS-CoV. SL-CoVs display greater genetic variation than SARS-CoVs isolated from humans or civets. SARS-CoVs nestle phylogenetically within the spectrum of SL-CoVs, indicating that the virus responsible for the SARS outbreak was a member of this new coronavirus group, tentatively named the SARS cluster of coronaviruses (SCCoVs).

Conclusions: Serologic and molecular surveys revealed the presence of SCCoVs in different species of horseshoe bats covering a wide geographic area in China. Bats are found in live animal markets across China, are eaten by people, and their feces and other body parts used in traditional medicine. We hypothesize that SCCoVs spilled over from this natural reservoir to civets and other immunologically naïve species in the wild animal markets, leading to a cycle of infection in traded wildlife, and thence to humans.

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Echinococcosis in Tibetan Populations of Western Sichuan Province, China

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Background: Human cystic echinococcosis (CE), caused by infection with the larval stage of *Echinococcus granulosus*, and alveolar echinococcosis (AE), caused by infection with the larval stage of *E. multilocularis*, are two of the most pathogenic zoonotic parasitic helminthic infections of humans in the Northern Hemisphere. Human CE occurs worldwide in association with livestock herding, within which the main dog-sheep cycle for *E. granulosus* is transmitted. Human AE is a much rarer parasitic infection and is primarily transmitted between foxes and small mammals in wildlife cycle.

Methods: We screened 3,199 people from Shiqu County, Ganze Tibetan Autonomous Prefecture, Sichuan Province, China, for abdominal echinococcosis (hydatid disease) by portable ultrasound combined with specific serodiagnostic tests.

Results: Both CE and AE were co-endemic in these populations with the highest village co-endemic prevalence values recorded anywhere in the world: 12.9% were infected with one or the other form (6.8% CE and 6.1% AE). The prevalence of both CE and AE was significantly higher in females than in males and increased with the age of the person screened. Pastoral Tibetan herdsmen were at highest risk for infection (prevalence 19.0%). Prevalence of CE varied in 5 townships from 0% to 12.1%, while AE prevalence ranged from 0% to 14.3%. Risk factors associated with both infections included the number of owned dogs, frequency of contact with dogs, and sources of drinking water.

Conclusions: Both CE and AE disease should be considered as a public health priority in the pastoral communities of the eastern Tibetan Plateau.

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. 3. 25	該当なし	

一般的名称	解凍人赤血球濃厚液	研究報告の公表状況	Wang QH, Han MG, Cheetham S, Souza M, Funk JA, Saif LJ. Emerg Infect Dis. 2005 Dec;11(12):1874-81.	公表国
販売名（企業名）	解凍赤血球濃厚液「日赤」（日本赤十字社） 照射解凍赤血球濃厚液「日赤」（日本赤十字社）			米国

研究報告の概要

○ブタ・ノロウイルスはヒト・ノロウイルスに関連していた

日本及びヨーロッパにおける成体ブタからの遺伝子型IIグループ（GII）ノロウイルス（NoV）のRNA検出及び米国のブタからのGII NoV抗体の検出は、米国のブタでNoVが検出されていないにも関わらず、ブタNoVのヒトへの感染に関して公衆衛生上の懸念をもたらしている。ブタNoVを検出し、ウイルスの遺伝的多様性及びヒトNoVとの関連性を調査するため、健常な米国の成体ブタ由来の糞便275サンプルを、カリシウイルスuniversal primerを用いた逆転写ポリメラーゼ連鎖反応によってスクリーニングした。6検体がNoV陽性であった。ブタNoVの5検体の3'末端における3kbの配列解析に基づき、GIIの遺伝子型3種と組換型と思われる1種が同定された。ブタNoVの遺伝子型のうち種は、遺伝的抗原的にヒトNoVと関連性があり、ノトバイオートのブタで複製された。こうした結果は、無症候感染の成体ブタが新種のヒトNoVの宿主となりうるか、あるいはブタ/ヒトのGII組換え型が出現する可能性を示唆するものである。

報告企業の意見	今後の対応
ブタNoVの遺伝子型のうち1種は、遺伝的抗原的にヒトNoVと関連性があったとの報告である。	今後も引き続き、新興・再興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

解凍赤血球濃厚液「日赤」
照射解凍赤血球濃厚液「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

(5)

RESEARCH

Porcine Noroviruses Related to Human Noroviruses

Qiu-Hong Wang,* Myung Guk Han,* Sonia Cheetham,* Menira Souza,*
Julie A. Funk,† and Linda J. Saif*

Detection of genogroup II (GII) norovirus (NoV) RNA from adult pigs in Japan and Europe and GII NoV antibodies in US swine raises public health concerns about zoonotic transmission of porcine NoVs to humans, although no NoVs have been detected in US swine. To detect porcine NoVs and to investigate their genetic diversity and relatedness to human NoVs, 275 fecal samples from normal US adult swine were screened by reverse transcription–polymerase chain reaction with calicivirus universal primers. Six samples were positive for NoV. Based on sequence analysis of 3 kb on the 3′ end of 5 porcine NoVs, 3 genotypes in GII and a potential recombinant were identified. One genotype of porcine NoVs was genetically and antigenically related to human NoVs and replicated in gnotobiotic pigs. These results raise concerns of whether subclinically infected adult swine may be reservoirs of new human NoVs or if porcine/human GII recombinants could emerge.

Noroviruses (NoVs) (family *Caliciviridae*, genus *Norovirus*) cause diarrhea in humans and animals (1–3). The NoV genome is 7.3–7.7 kb long with 3 open reading frames (ORFs) encoding a polyprotein that undergoes protease processing to produce several nonstructural proteins, including an RNA-dependent RNA polymerase (RdRp), a major capsid protein (VP1, capsid), and a minor capsid protein (VP2) (1,4,5). The capsid protein contains a conserved shell (S) and hypervariable protruding (P) domains (6). Noroviruses are genetically diverse and make up 27 genotypes within 5 genogroups, GI/1–8, GII/1–17, GIII/1–2, GIV, and GV, based on the capsid genes of 164 strains (7). Human NoVs cause an estimated 23 million cases of illness annually in the United States (8) and >90% of nonbacterial epidemic gastroenteritis worldwide (1). The low infectious dose, environmental resistance, strain diversity, shedding from asymptomatic persons, and varied transmission vehicles render human NoVs highly contagious.

Norovirus RNA was detected by reverse transcription–polymerase chain reaction (RT-PCR) in 4 of 1,017 normal slaughtered pigs in Japan (9) and in 2 of 100 pooled pig fecal samples in the Netherlands (10). These porcine NoVs (Sw43/97/JP, Sw918/97/JP, and 34/98/NET) are genetically similar and are classified into GII (9,10), like most epidemic human NoVs (11–13). Also, the virus-like particles (VLPs) of Sw918 strain cross-react with antibodies against human GII but not GI NoVs (14). The close genetic and antigenic relationships between human and porcine NoVs raise public health concerns regarding their potential for zoonotic transmission and as reservoirs for emergence of new epidemic human strains.

Farkas et al. (14) reported that US swine sera react with Po/NoV/GII/Sw918 strain, but no direct detection of NoV from US swine has been reported. To detect porcine NoVs and assess their genetic diversity and relatedness to human NoVs, we screened 275 pig fecal samples from US swine by RT-PCR with a calicivirus universal primer pair p290/110 targeting the RdRp region (15,16), followed by sequencing the 3 kb on the 3′ end of the genome for 5 NoV strains. Gnotobiotic pigs were inoculated with porcine NoVs to examine their infectivity and to produce convalescent-phase antiserum for antigenic analysis.

Materials and Methods

Fecal samples (N = 275) were collected from December 2002 to June 2003 from finisher (10–24 weeks of age) pigs and gestating sows (≥1 year of age) from 3 Ohio swine farms (10, 60, and 32 samples), 1 Ohio slaughterhouse (83 samples), 1 Michigan swine farm (61 samples), and 2 North Carolina swine farms (8 and 21 samples). Fresh fecal samples were collected from individual pigs, placed into sterile containers, and stored frozen.

Sample RNA was extracted from 10% to 20% of fecal suspensions in sterile Eagle minimal essential medium

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(EMEM, Invitrogen, Carlsbad, CA, USA) by using Trizol LS (Invitrogen). For some samples, RNA was concentrated and purified by using QIAamp Viral RNA Mini kit (Qiagen, Valencia, CA, USA).

RT-PCR was performed separately by using primer pair p290 (5′-GATTACTCCAAGTGGGACTCCAC-3′) (15) and p110 (5′-ACDATYTCATCATCACCATA-3′) (16) as previously described (15) but at 48°C for annealing (317 bp for NoV or 329 bp for sapovirus). To amplify the 3-kb 3′ end fragment, cDNA was synthesized by SuperScript III First-Strand cDNA synthesis kit (Invitrogen) with primer VN_3T_{20} (5′-GAGTGACCGCGGCCGCT$_{20}$-3′). PCR was then performed with TaKaRa Ex Taq polymerase (TaKaRa Mirus Bio, Madison, WI, USA) with primers p290 and VN_3T_{20}. Quantitative (endpoint titration) RT-PCR (17) was performed with primer pair PNV7 (5′-AGGTGGTGGCCGAGGAYCTCCT-3′) and PNV8 (5′-TCACCATAGAAGGARAAGCA-3′) targeting the RdRp (211 bp) of QW101 strain.

RT-PCR products were purified with the QIAquick Gel Extraction kit (Qiagen) before cloning into pCR2.1-TOPO (T/A) or PCR XL cloning kit (Invitrogen). Five clones of each sample were sequenced. DNA sequencing was performed with BigDye Terminator Cycle and 3730 DNA Analyzer (Applied Biosystems, Foster City, CA, USA).

Sequence editing was performed by Lasergene software package (v5, DNASTAR Inc., Madison, WI, USA). The Basic Local Alignment Search Tool (BLAST, http://www.ncbi.nlm.nih.gov/BLAST) was used to find homologous hits. Multiple sequence alignment was performed with ClustalW (v1.83) at DNA Data Bank of Japan (http://www.ddbj.nig.ac.jp). Phylogenetic and bootstrap (1,000 replicates) analyses were conducted by using MEGA (v2.1) (18). Identification of recombinants was performed by using the Recombinant Identification Program (RIP, http://hivweb.lanl.gov/RIP/RIPsubmit.html) (19). The classification and GenBank accession numbers of NoVs are listed in Table 1.

Four gnotobiotic pigs were maintained and euthanized as previously described (25,26). The inoculate was a 20% fecal filtrate (0.2 μm) in EMEM of the QW126 or QW144 (QW101-like, GII-18) strains or EMEM only (2 negative control pigs). One pig was inoculated with QW126 orally and intranasally at 9 days of age, and convalescent-phase antiserum LL616 was collected at postinoculation day (PID) 26. A second pig was inoculated with QW144 orally at 35 days of age and euthanized at PID 5.

Immune electron microscopy (IEM) was performed as described previously (27). For enzyme-linked immunosorbent assay (ELISA), the recombinant baculovirus-expressed human NoV VLPs and rotavirus VP2 and VP6 (2/6)-VLPs (negative control) (28) were CsCl-gradients purified. We coated 96-well microplates with VLPs (200 ng/well) in carbonate buffer (pH 9.6) and blocked with 5% nonfat dry milk in phosphate-buffered saline (PBS)-Tween 20 (0.05%). Serially diluted serum samples that included positive and negative controls were added to duplicate positive- and negative-coated wells, and the plates were incubated. After washing, horseradish peroxidase (HRP)-labeled goat anti-pig immunoglobulin G (IgG) (H + L) for pig sera or goat anti-human IgG + IgA + IgM (H + L) (KPL, Gaithersburg, MD, USA) for human serum was added. After incubation and washing, the substrate 3,3′,5,5′-tetramethylbenzidine was added. The cutoff value was the mean absorbance of the negative coatings multiplied by 2.

Western blot was performed as described previously (29). Nitrocellulose membranes were incubated with pig convalescent-phase antiserum LL616 against porcine GII-18 NoV or negative control serum in PBS containing 4% nonfat dry milk followed by goat anti-pig IgG (H + L)-HRP conjugate.

Results

Porcine NoVs were classified into 3 genotypes within GII based on the complete capsid sequences: 1 genotype with prototype Japanese strains Sw43 and Sw918 and 2 new genotypes. A total of 19 of 275 samples showed a potential positive band after agarose gel electrophoresis of the RT-PCR products of primer pair p290/110. Fourteen samples representative of each potentially positive farm or the slaughterhouse were sequenced. After performing BLAST search, we identified 6 NoVs (QW48, Michigan farm A; QW101, QW125, and QW126, Ohio farm B; and QW170 and QW218, Ohio slaughterhouse), 3 sapoviruses, and 5 sequences that had no significant hit in the database. Because the QW126 shared 99% nucleotide (nt) identity with the QW101 and QW125 strains in the 274-nt RdRp region, it was not sequenced further.

We sequenced the 3-kb 3′ end of the genome containing the partial RdRp, VP1 and VP2 genes, and the 3′ untranslated region of the 5 strains. The porcine NoVs represented 3 distinct clusters: 1) Sw43, Sw918, and QW48; 2) QW101 and QW125; and 3) QW170 and QW218, on the basis of the size of each gene and the ORF1-ORF2 overlap region (Table 2). Across the 3 kb, the QW101 and QW125 strains and the QW170 and QW218 strains shared 99% nt identity.

The amino acid identity of the predicted complete and S and P domains of the capsid protein of the 5 porcine NoVs, the previously reported porcine NoVs (Sw43 and Sw918), and representative human, bovine, and murine NoV strains is summarized in Table 3. In the complete capsid, the QW48 strain was most closely related to the porcine NoV prototype Sw43 strain (98% amino acid identity); the QW170 and QW218 strains shared the highest

amino acid identities (81%) to porcine Sw43 and Sw918 strains; the QW101 and QW125 strains showed the highest amino acid identity to human GII-3/Mexico (71.4%), then to human GII-6/Baltimore (71.0%), porcine QW218 (71.0%), and porcine Sw43 (70.6%) strains. The S and P domains of these NoVs showed similar relationships. A neighbor-joining phylogenetic tree based on the amino acid sequences of the complete capsids (Figure 1) showed that QW48 grouped with Sw43 and Sw918 strains into GII-11 and that QW170 and QW218 formed a new

Table 1. Classification and GenBank accession numbers of norovirus (NoV) strains used for sequence analysis*

Strain	Genus/genogroup-genotype	Abbreviation	GenBank accession no.
Hu/Norwalk/68/US	NoV/GI-1	Norwalk	M87661
Hu/Hawaii/71/US	NoV/GII-1	Hawaii	U07611
Hu/Melksham/89/UK	NoV/GII-2	Melksham	X81879
Hu/Snow Mountain/76/US	NoV/GII-2†	Snow Mountain	AY134748
Hu/Mexico/89/MX	NoV/GII-3	Mexico	U22498
Hu/Toronto/91/CA	NoV/GII-3	Toronto	U02030
Hu/SaitamaU18/97-99/JP	NoV/GII-3	SaitamaU18	AB039781
Hu/SaitamaU201/98/JP	NoV/GII-3	SaitamaU201	AB039782
Hu/Arg320/ARG	NoV/GII-3†	Arg320	AF190817
Hu/Camberwell/101922/94/AUS	NoV/GII-4	Camberwell	AF145896
Hu/Lordsdale/93/UK	NoV/GII-4	Lordsdale	X86557
Hu/Bristol/93/UK	NoV/GII-4	Bristol	X76716
Hu/MD145-12/87/US	NoV/GII-4	MD145	AY032605
Hu/Farmington Hills/02/US	NoV/GII-4	Farmington Hills	AY502023
Hu/Langen1061/02/DE	NoV/GII-4	Langen	AY485642
Hu/Hillingdon/93/UK	NoV/GII-5	Hillingdon	AJ277607
Hu/New Orleans 306/94/US	NoV/GII-5	New Orleans	AF414422
Hu/Baltimore/274/1993/US	NoV/GII-6	Baltimore	AF414408
Hu/SaitamaU3/97/JP	NoV/GII-6	SaitamaU3	AB039776
Hu/SaitamaU4/97/JP	NoV/GII-6	SaitamaU4	AB039777
Hu/SaitamaU16/97/JP	NoV/GII-6	SaitamaU16	AB039778
Hu/SaitamaU17/97/JP	NoV/GII-6	SaitamaU17	AB039779
Hu/Seacroft/90/UK	NoV/GII-6†	Seacroft	AJ277620
Hu/Leeds/90/UK	NoV/GII-7	Leeds	AJ277608
Hu/Gwynedd/273/94/US	NoV/GII-7	Gwynedd	AF414409
Hu/Amsterdam/98-18/98/NET	NoV/GII-8	Amsterdam	AF195848
Hu/SaitamaU25/97-99/JP	NoV/GII-8	SaitamaU25	AB039780
Hu/VA97207/97/US	NoV/GII-9‡	VA97207	AY038599
Hu/NLV/Erfurt/546/00/DE	NoV/GII-10	Erfurt	AF427118
Hu/Mc37/00-01/THA	NoV/GII-10†	Mc37	AY237415
Po/Sw43/97/JP	NoV/GII-11	Sw43	AB074892
Po/Sw918/97/JP	NoV/GII-11	Sw918	AB074893
Po/MI-QW48/02/US	**NoV/GII-11**	**QW48**	**AY823303**
Hu/Gifu/96/JP	NoV/GII-12‡	Gifu	AB045603
HU/Wortley/90/UK	NoV/GII-12†	Wortley	AJ277618
Hu/SaitamaU1/97-99/JP	NoV/GII-12†	SaitamaU1	AB039775
Hu/Fayetteville/98/US	NoV/GII-13	Fayetteville	AY113106
Hu/M7/99/US	NoV/GII-14	M7	AY130761
Hu/J23/99/US	NoV/GII-15	J23	AY130762
Hu/Tiffin/99/US	NoV/GII-16	Tiffin	AY502010
Hu/Neustrelitz260/00/DE	NoV/GII-16	Neustrelitz	AY772730
Hu/CS-E1/02/US	NoV/GII-17	CS-E1	AY502009
Po/OH-QW101/03/US	**NoV/GII-18**	**QW101**	**AY823304**
Po/OH-QW125/03/US	**NoV/GII-18**	**QW125**	**AY823305**
Po/OH-QW170/03/US	**NoV/GII-19‡**	**QW170**	**AY823306**
Po/OH-QW218/03/US	**NoV/GII-19‡**	**QW218**	**AY823307**
Bo/Newbury-2/76/UK	NoV/GIII-2	Newbury-2	AF097917
Hu/Alphatron/98-2/98/NET	NoV/GIV	Alphatron	AF195847
Mu/MNV-1/03/US	NoV/GV	MNV-1	AY228235

*Classification is based on the capsid gene sequences. The 5 porcine NoV strains sequenced in this study are in boldface.
†Previously reported recombinants (20–24).
‡Potential recombinants found in this study.

Table 2. Sizes of the putative capsid protein VP1 and the minor capsid protein VP2, the overlap regions, and the 3′ UTR of GII NoV*

Species/genogroup-genotype/strain	ORF1-ORF2 overlap (nt)	VP1 (aa)	ORF2-ORF3 overlap (nt)	VP2 (aa)	3′ UTR (nt)
Po/GII-11/Sw43	17	547	NA	NA	NA
Po/GII-11/Sw918	17	547	NA	NA	NA
Po/GII-11/QW48	17	547	1	253	57
Po/GII-18/QW101	20	557	1	275	48
Po/GII-18/QW125	20	557	1	275	48
Po/GII-19/QW170	17	548	1	254	51
Po/GII-19/QW218	17	548	1	254	51
Hu/GII-1/Hawaii	20	535	1	259	42
Hu/GII-2/Snow Mountain	20	542	1	259	45
Hu/GII-3/SaitamaU18	20	548	1	254	37
Hu/GII-4/MD145	20	539	1	268	46
Hu/GII-5/New Orleans	20	540	1	258	35
Hu/GII-6/SaitamaU3	20	550	1	259	54
Hu/GII-7/Gwynedd	20	540	1	257	68
Hu/GII-8/SaitamaU25	20	537	1	257	53
Hu/GII-9/VA97207	20	537	1	257	51
Hu/GII-10/Mc37	20	548	1	258	34
Hu/GII-12/SatamaU1	20	535	1	259	50
Hu/GI-1/Norwalk	17	530	1	212	66

*UTR, untranslated region; NoV, norovirus; ORF, open reading frame; nt, nucleotide; aa, amino acid; NA, not available.

genotype (GII-19), which was closer to porcine than to human strains. However, QW101 and 125 formed a new genotype (GII-18) between human and porcine GII NoVs.

Further analysis of the predicted C-terminal ≈260 amino acids of the RdRp region (Figure 2) showed similar grouping results for QW48, QW101, and QW125 strains but different for QW170 and QW218 strains, which were in the same cluster (GII-11) as Sw43, Sw918, and QW48 in the RdRp region. This finding suggested that a recombination event occurred between QW170/218-like and Sw43-like NoVs. The complete VP2 sequences of representative strains were also analyzed (data not shown). Results were similar to those of the capsid sequence classification.

A potential recombination event occurred between QW170/218-like and Sw43-like strains. To examine where the recombination occurred, we performed RIP analysis by placing the 3′-end RdRp and the capsid sequence of QW170 or QW218 as a query sequence and the corresponding sequences of Sw43 and QW101 as background sequences. The resulting diagram (Figure 3A) showed that QW170 had high similarity to Sw43 in the RdRp but not in the capsid region. This abrupt change happened in the RdRp-capsid junction region. Therefore, we performed sequence alignments of the RdRp-capsid junction of NoVs, including the calicivirus genomic-subgenomic conserved 18-nt motif (20) (Figure 3B). Between Sw43, QW170, and QW218, all 18 nt were identical, but identities decreased downstream of this motif. QW170 and QW218 grouped with Sw43 with a high bootstrap value of 95 in the RdRp tree (Figure 2), whereas they segregated from Sw43 with the highest bootstrap value of 100 in the capsid tree (Figure 1). We could not clarify which was the parent or progeny strain.

The porcine NoVs replicated in gnotobiotic pigs. Two pigs were inoculated with QW101-like GII-18 porcine NoVs (QW126 and QW144 strains) to verify their replication in pigs as confirmed by quantitative RT-PCR and IEM and to produce convalescent-phase serum to examine antigenic reactivity with human NoVs. These 2 strains were confirmed as QW101-like porcine NoVs in both the RdRp (169-nt) and the capsid S domain (363-nt) regions by sequence analysis of the RT-PCR products (Q.H. Wang and L.J. Saif, unpub. data). They shared 99% and 100% amino acid identities to the QW101 strain in the 2 regions, respectively. Porcine NoV shedding, assessed by quantitative RT-PCR with primer pair PNV7/8, was detected at PID 3–5 (euthanized) after QW144 exposure, coincident

Table 3. Percentage amino acid identities of noroviruses within the capsid region

	Complete capsid (S domain, P domain)					
Strain	Po/GII*	Hu/GII†	Hu/GI/ Norwalk	Bo/GIII/ Newbury-2	Hu/GIV/ Alphatron	Mu/GV/MNV-1
QW48	96–98 (100, 94–97)	63–71 (77–85, 53–63)	43 (59, 36)	45 (62, 36)	53 (71, 42)	39 (58, 29)
QW101, QW125	70–70.6 (83, 63)	61–71.4 (77–86, 51–64)	42 (59, 35)	45 (62, 38)	54 (71, 44)	39 (58, 28)
QW170, QW218	81 (90, 74)	62–69 (77–82, 52–62)	43 (59, 36)	45 (61, 37)	53 (72, 40)	39 (60, 27)

*Includes Sw43 and Sw918 strains.
†Includes Hawaii, Snow Mountain, Mexico, MD145, New Orleans, Baltimore, Gwynedd, Amsterdam, VA97207, Erfurt, Gifu, Fayetteville, M7, J23, and Neustrelitz strains.

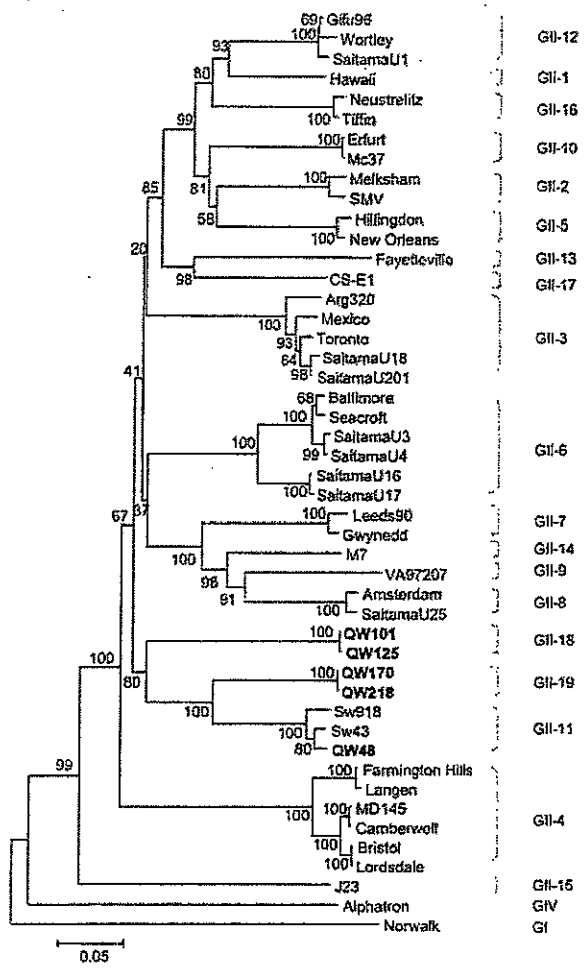

Figure 1. Neighbor-joining phylogenetic tree of genogroup II noroviruses (NoVs) based on the complete capsid region. The 5 newly identified porcine NoV strains are in **boldface**. Genogroups (G) and genotypes (numbers after G) are indicated. The human NoV GI-1/Norwalk and GIV/Alphatron strains were used as outgroup controls.

with mild diarrhea. The RT-PCR–detectable units of the rectal swab RNA increased from negative at PID ≤ 2, 10^3 at PID 3–4, and 10^4 at PID 5 (large intestinal contents). Norovirus shedding was detected only at PID 5 without diarrhea after QW126 exposure. Examination of the intestinal contents of the pig inoculated with QW144 by IEM with pig convalescent-phase antiserum LL616 showed clumps of ≈32-nm NoV particles (Figure 4). The 2 control pigs had no virus shedding or diarrhea. Detailed studies of the pathogenesis of porcine NoVs in gnotobiotic pigs are in progress (S. Cheetham and L.J. Saif, unpub. data).

Antisera to QW101-like (QW126) porcine NoVs cross-reacted with VLPs of human GII NoVs in ELISA and Western blot. In ELISA (Table 4), the pig convalescent-phase antiserum (LL616) to QW101-like porcine NoV QW126 strain showed higher titers (1:400–1:800) to GII-3/Toronto, GII-4/MD145, GII-4/HS66, and GII-6/Florida strains; a lower titer (1:100) to GII-1/Hawaii strain; and lowest titer (1:10) to GI-3/Desert Shield strain. In Western blot (Figure 5), the capsid proteins (59–60 kDa) of Toronto, MD145, HS66, and Florida strains, but not the Hawaii and Desert Shield strains, were detected by pig antiserum LL616 but not the negative control serum (data not shown). Thus, 1-way antigenic cross-reactivity exists between human NoV antigens and porcine NoV (GII-18) antiserum, with moderate cross-reactivity to human NoVs GII-3, 4, and 6; low cross-reactivity to GII-1; and very low cross-reactivity to GI-3.

Discussion

All porcine NoVs were detected from pigs without clinical signs (9,10). Subclinically infected pigs may be natural reservoirs for NoVs, and because porcine GII NoVs are genetically and antigenically related to human NoVs, concerns exist about their zoonotic potential. Whether human NoV strains similar to the QW101-like porcine NoVs circulate among people with occupational exposure to pigs is

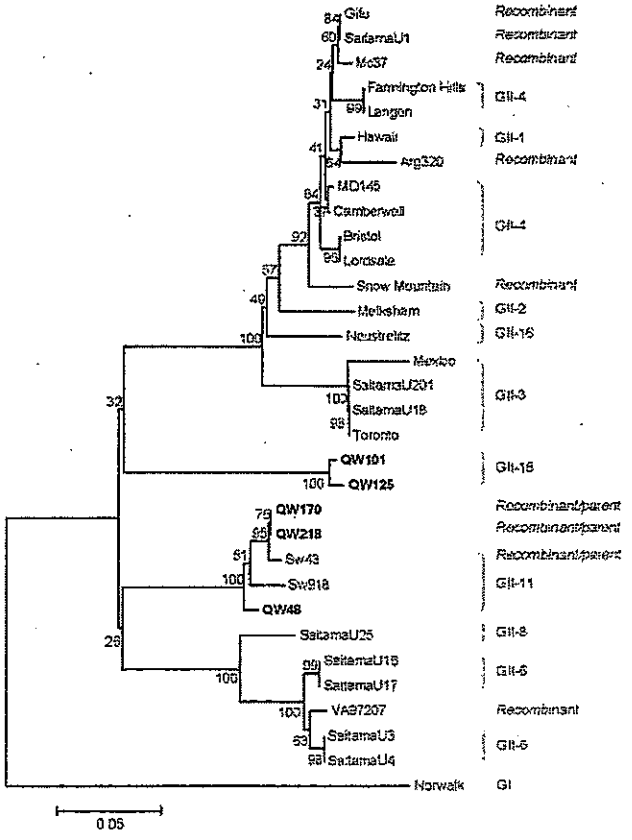

Figure 2. Neighbor-joining phylogenetic tree of genogroup II noroviruses (NoVs) based on the partial RNA-dependent RNA polymerase region (C-terminal 260–266 amino acids). The 5 newly identified porcine NoV strains are in **boldface**. Genogroups (G) and genotypes (numbers after G) are indicated. The human NoV GI-1/Norwalk and strain was used as outgroup control.

unknown, but such studies could provide information on the zoonotic potential of these porcine NoVs.

The RdRp-capsid junction region of NoVs contains a highly conserved 18-nt motif in genomic and subgenomic RNA that is believed to be a transcription start signal (1,20). All 18 nt were identical within each genogroup except for the Hu/GII/J23, Po/GII/QW101, and Po/GII/QW125 strains (Figure 3B, sequence alignments on other GI and GIII strains are not shown). This finding suggests that homologous recombination may occur within this motif between NoVs of different genotypes within the same genogroup. Recombinant human GII NoVs have been reported previously (20–24). To our knowledge, this study is the first identification of a potential recombinant between pig NoVs. At present, NoV recombinants have been detected exclusively between viruses within the same genogroup and within the same host species, but few animal NoVs have been sequenced (RdRp and capsid) for comparative analysis, especially those from animals in developing countries, where humans and animals may be in close contact.

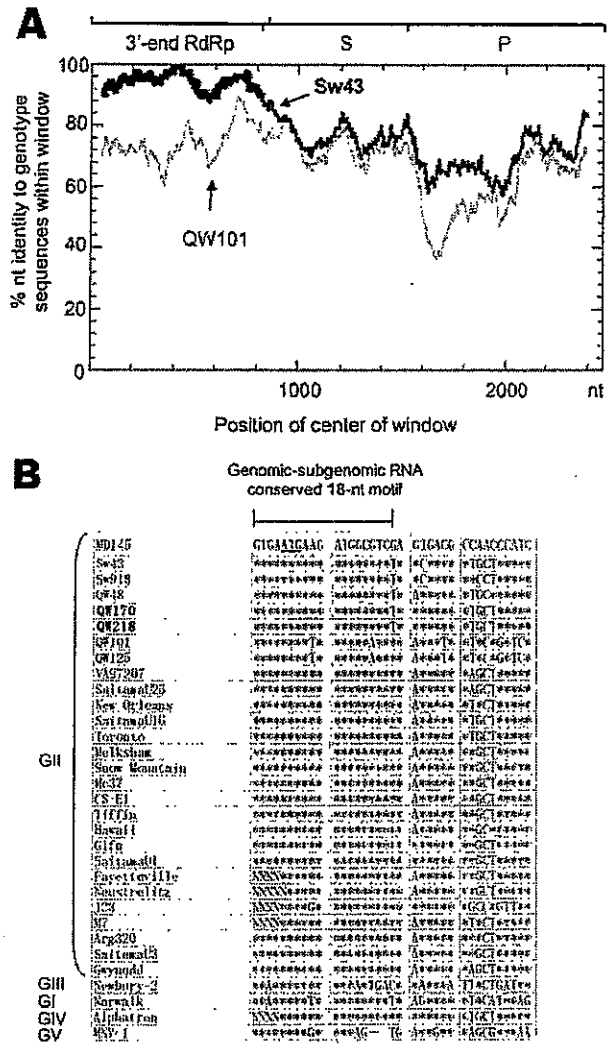

Figure 3. Identification of a potential recombination event between QW170 and Sw43 strains. A) Recombination Identification Program analysis of QW170 strain. At each position of the window, the query sequence (QW170) was compared to each of the background genotype representatives (GII-11/Sw43 and GII-18/QW101). When the query sequence is similar to the background sequences, the homologous regions are indicated as thick lines on the plot. Analysis parameters were window size of 100 and significance of 90%. The nucleotide positions of the 3′-end RNA-dependent RNA polymerase (RdRp) and the shell (S) and protruding (P) domains of the capsid protein are indicated. B) Sequence alignments of the RdRp-capsid junction region of noroviruses (NoVs). The genomic and subgenomic conserved 18-nucleotide (nt) motif is indicated by a horizontal line with 2 vertical bars. Asterisks indicate the identical residues to the sequence of the first line. Dashes represent gaps. The letter N indicates missing data on the residue. The start codon of open reading frame ORF 2 is underlined. Five NoV genogroups are indicated.

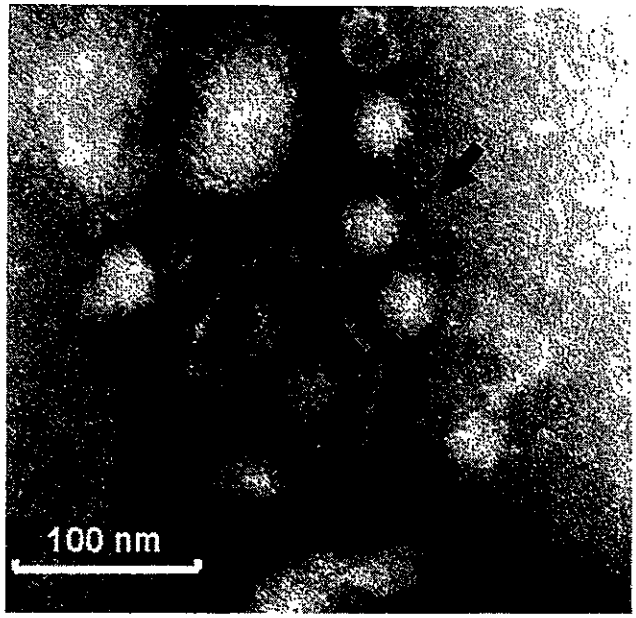

Figure 4. Immune electron micrograph of porcine noroviruses (NoVs). The diluted intestinal contents of a gnotobiotic pig euthanized on postinoculation day 5 to QW101-like porcine NoVs (QW144) were incubated with convalescent-phase serum LL616 from another gnotobiotic pig inoculated with QW101-like porcine NoVs (QW126) and visualized by negative staining with 3% phosphotungstic acid. The arrow indicates a small clump of NoV-like particles.

The QW101-like porcine NoVs replicated in gnotobiotic pigs with fecal shedding, documented by quantitative RT-PCR and IEM. No cell culture system or animal disease models are available for human NoVs, which impedes the study of their pathogenesis, replication strategies, host immune responses, and preventive approaches. The infection of pigs with porcine NoVs may provide a new infection or disease model to study NoV infections.

Table 4. Antigenic cross-reactivity between human GII NoV antigens (VLPs) and a pig convalescent-phase antiserum against porcine GII NoVs, as determined by ELISA*

Antiserum	ELISA antibody titer with each VLP antigen (genogroup-genotype)					
	Hawaii (GII-1)	Toronto (GII-3)	MD145 (GII-4)	HS66 (GII-4)	Florida (GII-6)	Desert Shield (GI-3)
HS66CS (positive control): human convalescent-phase antiserum to human HS66 (GII-4)	1:25,600	1:6,400	1:25,600	1:25,600	1:6,400	1:6,400
LL616: pig convalescent-phase antiserum to porcine QW126 (QW101-like, GII-18)†	1:100	1:800	1:400	1:400	1:400	1:10
LL368 (negative control): preinoculation serum‡	<1:10	<1:10	<1:10	<1:10	<1:10	<1:10
MM982 (negative control): preinoculation serum‡	<1:10	<1:10	<1:10	<1:10	<1:10	<1:10

*NoV, norovirus; VLP, viruslike particle; ELISA, enzyme-linked immunosorbent assay.
†The QW126 shared 99% and 100% amino acid identities to the QW101 strain (GII-18) for a 169-bp segment in the RNA-dependent RNA polymerase region and a 363-bp segment in the capsid region, respectively.
‡LL368 and MM982 were sera from 2 gnotobiotic pigs before inoculation with porcine NoVs.

In this study, 1-way antigenic cross-reactivity occurred between antiserum to QW101-like porcine NoVs and the capsid proteins of human NoVs, with highest cross-reactivity to GII-3, 4, and 6 NoVs. This finding coincides with the finding that the QW101 strain shares high amino acid identity with GII-3 (71%), GII-6 (71%), and GII-4 (63%) NoVs.

In summary, 3 genotypes of porcine NoVs were detected in US swine. One genotype (QW101-like, GII-18) was genetically and antigenically most closely related to human GII NoVs. Potential recombinant porcine NoV strains were identified. The QW101-like NoVs infected gnotobiotic pigs, and NoV particles were evident in intestinal contents. These results raise questions of whether pigs may be reservoirs for emergence of new human NoVs or if porcine/human GII recombinants could emerge.

Acknowledgments

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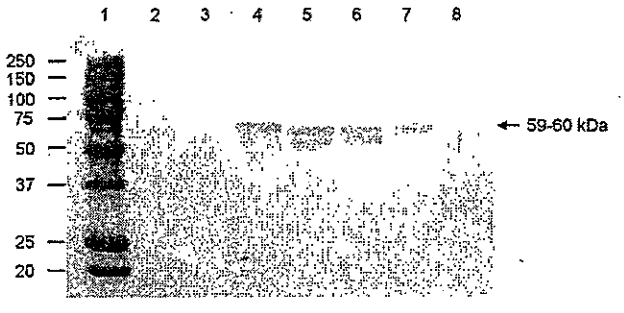

Figure 5. Antigenic cross-reactivity between human genogroup (G) II norovirus (NoV) capsid proteins and a pig convalescent-phase antiserum (LL616) against porcine QW101-like (GII-18) NoV was determined by Western blot. The CsCl-gradient purified viruslike particles (1,250 ng) were separated by sodium dodecyl sulfate 10% polyacrylamide gel electrophoresis, blotted onto nitrocellulose membranes, and tested with LL616. The sucrose-cushion (40%, wt/vol) purified Sf9 insect cell proteins acted as a negative control (lane 8). Lane 1, molecular weight marker (kDa); lanes 2–7, Hu/GI-3/Desert Shield, Hu/GII-1/Hawaii, Hu/GII-3/Toronto, Hu/GII-4/MD145, Hu/GII-4/HS66, and Hu/GII-6/Florida, respectively.

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別紙様式第2-1

No. 19

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. 4. 26	該当なし	
一般的名称	人全血液		研究報告の公表状況	H Cordel, I Quatresous, C Paquet, E Couturier. Eurosurveillance weekly release: 20 April 2006	公表国 フランス	
販売名(企業名)	人全血液CPD「日赤」(日本赤十字社) 照射人全血液CPD「日赤」(日本赤十字社)					

研究報告の概要

○フランス本土におけるチクングンヤの輸入例 2005年4月～2006年2月
導入:2006年4月9日時点で、241,000のチクングンヤの症例がレユニオン島から、5,339例がマヨット島から報告されている。レユニオンから本土への渡航者は毎年30万人に上り、媒介蚊のヒトスジシマカがフランス及びイタリア北西部でも発見されているため、本土での流行が懸念される。
方法:チクングンヤの血清学検査を行っているフランス本土の検査施設のデータを分析した。患者と施設の郵便番号、患者の年齢、性別、検体採取の日付を分析に使用した。患者の渡航日と発症日は得られなかった。輸入例は以下のように定義された。
・チクングンヤIgM抗体陽性及び/あるいはPCR陽性及び/あるいはウイルス培養陽性
・患者がフランス本土在住か否かを問わず、本土で検体が採取された
結果:輸入例307例が検出された。平均年齢は47歳(7歳～81歳)で、男女比は0.8:1だった。2005年4月～7月の輸入例数は毎月平均20例で、コモロ諸島での流行およびレユニオンでの流行の最初のピークと一致している。8月から11月にかけて症例数は減少したが、12月、特に最終週には著しく多くなり、2006年2月には131例が検出された。この傾向は、同時期のレユニオンでの流行とよく似ている。患者のほとんどはフランス南東部およびパリ在住だった。フランス本土内での感染が2006年3月に1例発生した。海外で感染した患者を担当した看護師が、3日後にチクングンヤの症状を発症した。看護師はインド洋への渡航歴はなく、血液の暴露による感染が疑われた。
考察:輸入例の患者は、ほとんどがフランス南東部のプロヴァンス・アルプ・コートダジュール地方の住民である。この地方、特にマルセイユ市にはコモロ系移民のコミュニティがあり、メンバーはよくコモロに渡航している。チクングンヤ感染は無症候あるいは軽症のことが多いため、診察や検査を受けておらず、感染が確認されていない患者も多いと考えられる。輸入例に重篤なものはなかったが、治療のために本土に移送されたレユニオンの患者には一部重症となったものもあり、劇症肝炎(急性肝不全)のために肝臓移植が必要となった症例もあった。

使用上の注意記載状況・その他参考事項等

人全血液CPD「日赤」
照射人全血液CPD「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
フランス領レユニオン島においてチクングンヤウイルス感染症が大流行しており、フランス本土においても患者を担当した看護師がチクングンヤの症状を発症したとの報告である。	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。

(6)

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Imported cases of chikungunya in metropolitan France, April 2005 - February 2006

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Introduction

By 9 April 2006, 241 000 cases of chikungunya had been reported on the island of Reunion, and 5339 cases on the island of Mayotte. The islands of Mauritius, Seychelles, Madagascar, and Comoros are also affected. Imported cases have been reported from a number of European countries [1].

Each year, about 300 000 people travel to Reunion from metropolitan France (mainland France and Corsica), and therefore the risk of an outbreak in metropolitan France must be considered, especially since the vector, the *Aedes albopictus* mosquito, has been detected in metropolitan France and in northwest Italy, near the borders with France, Spain and Switzerland. Its role in transmission of the virus depends on vectorial competence (intrinsic to the mosquito) and vectorial capacity (dependent on the environment).

In addition to mosquito surveillance, the number of imported human cases must be reported as accurately as possible, in order to assess the risk of transmission within mainland Europe.

Methods

Recent infection is likely if chikungunya IgM antibodies are detected in the five days after symptom onset, but the presence of antibodies does not necessarily mean that the patient is viraemic. In metropolitan France, serology is carried out by two private laboratories and the two national reference laboratories, which also perform PCR and viral culture. Data from laboratories from April 2005 to the end of February 2006 have now been analysed. Variables used were patient and laboratory postcodes, patient age, patient sex, and date of the blood sample. Data on the patients' dates of travel and illness onset were not available from the laboratory database.

An imported case was defined as:
- detection of IgM antibodies against chikungunya virus and/or positive PCR, and/or positive viral culture,
- sampled in metropolitan France, whether or not the patient lives in metropolitan France.

Results

From 1 April 2005 to 28 February 2006, 307 imported cases of chikungunya were identified in France. The mean patient age was 47 years (range: 7-81 years), and the male-female sex ratio was 0.8:1.

Between April and July 2005, an average of 20 imported cases was observed each month. These cases correspond to the outbreak in Comoros (over 5000 cases), and to the first peak of the Reunion outbreak (during week 19 of 2005). Incidence then decreased between August and November. The number of cases greatly increased in December 2005, particularly in the final week of that month, and 131 imported cases were identified in February 2006. This trend is similar to the epidemic curve of the Reunion outbreak where weekly incidence greatly increased at the end of December 2005 (Figures 1 and 2). Most of the cases imported to France have been in patients living in southeast France and the Paris region (Figure 3).

Figure 1. Temporal evolution of imported chikungunya infections into metropolitan France, by date of blood sample, April 2005 – February 2006

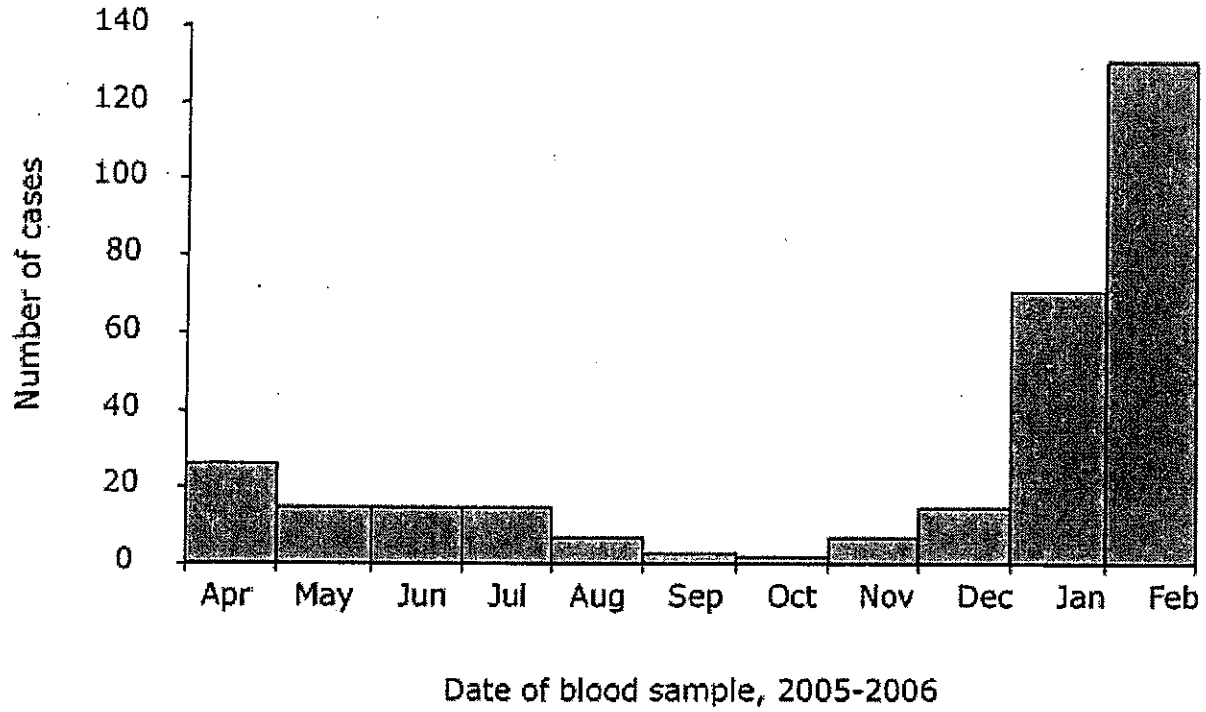


Figure 2. Chikungunya infections reported in Reunion, by date of illness onset, March 2005 – April 2006

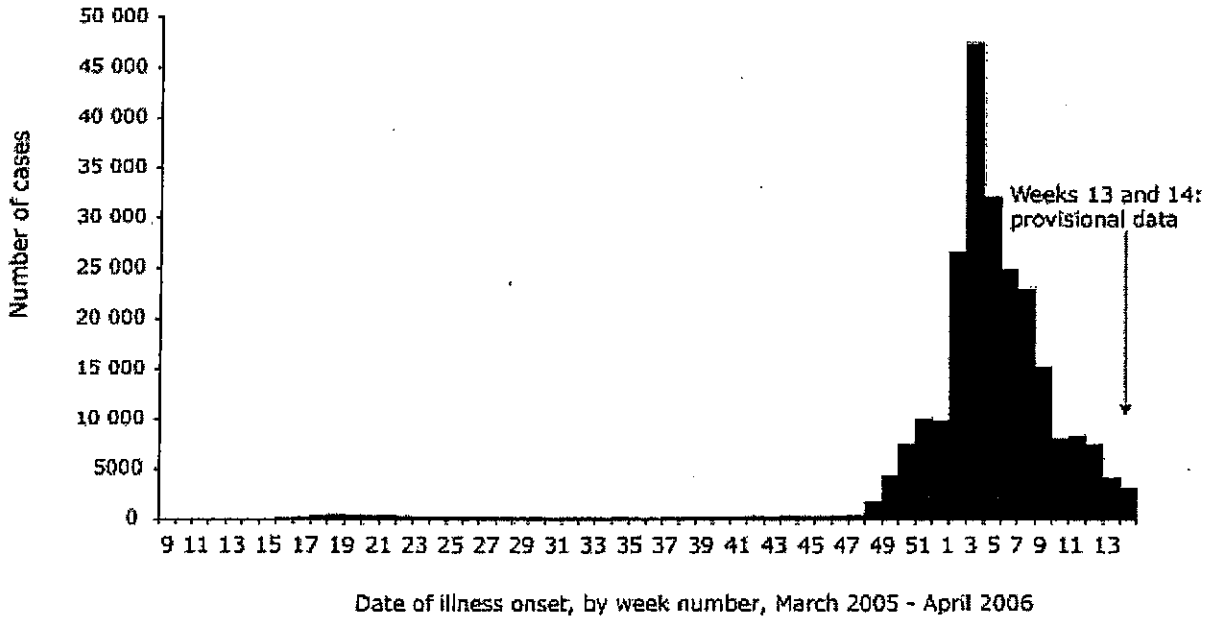
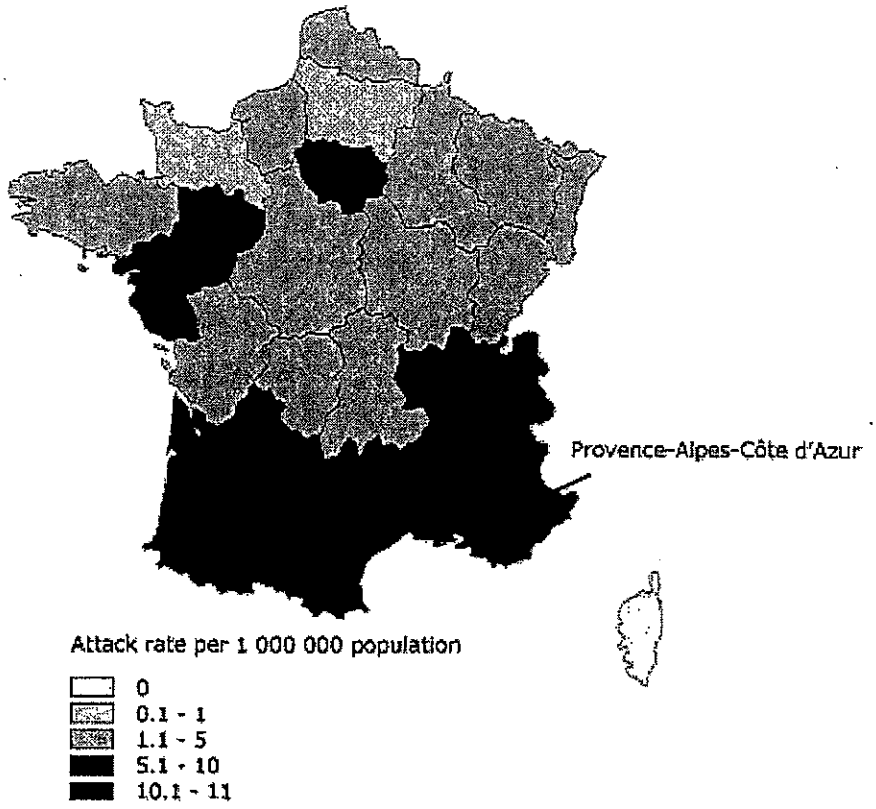


Figure 3. Attack rate per 1 000 000 population, by region, of imported chikungunya infections in metropolitan France, April 2005 – February 2006



An autochthonous case was reported in metropolitan France in March 2006. A nurse developed chikungunya fever (laboratory confirmed) three days after caring for a patient with an imported infection. The nurse had never travelled to the Indian Ocean, and investigation of this case has concluded that there was a probable blood exposure incident. Previous incidents involving transmission of chikungunya virus during laboratory procedures have been described [2,3,4].

Discussion

Most of the imported cases are in patients living in the Provence-Alpes-Côte d'Azur region in southeast France, which is home to a large Comorian community, particularly in the city of Marseille. Members of the community frequently travel to Comoros.

The imported cases reported here have been collated from laboratory data. Because chikungunya infections may be asymptomatic or have only mild clinical symptoms, it is likely that many or most of the people who have been ill with chikungunya in metropolitan France have not visited a doctor, and have not had their infections laboratory confirmed. Information on date of illness onset in relation to date of return to France would be a better indication of whether any of these patients had been viraemic when in metropolitan France, and thus present a risk for autochthonous transmission.

While none of the imported cases have been reported to be serious, some residents of Reunion who have become seriously ill with chikungunya on the island have been transferred to hospitals in metropolitan France for care. These patients include, for example, those who needed liver transplants to treat fulminant hepatitis (acute liver failure)

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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13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ポリエチレングリコール処理抗破傷風人免疫グロブリン ②乾燥抗破傷風人免疫グロブリン	研究報告の公表状況	Journal of Medical Primatology 2005; 34(5-6 Iss. S1): 333	公表国 アメリカ	
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研究報告の概要

サル泡沫状ウイルス（Simian foamy virus ；以下SFV）は、ヒト以外の様々な霊長動物に蔓延している。感染の様式は十分に研究されていないが、感染率が高いのは唾液によって感染が起こるためである。ヒトへの種の壁を超えての感染が中央アフリカのハンターに見つかり、感染した動物との職業上の偶発的接触による感染の可能性がある。感染すると長期間ウイルスが持続する。自然宿主での感染に比較して、SFVのヒトからヒトへの感染の証拠はこれまでない。しかし、かつてAIDSが流行したことに鑑み、たとえ自然宿主に疾患が無かったとしても、レトロウイルスの人獣共通感染症を防ぐための警戒を欠かないようにしなければならない。加えて、ヒトへの感染によってウイルスがさらに順応することは断固避けなければならない。最近、ヒトでのSFV感染が報告されたことから、血液ドナーによるSFV感染の可能性について懸念されている。SFVが血液によって感染するか否かを調査するため我々は、SFV陰性のアカゲザルに対して、生物学的且つ遺伝的に異なるSFVに自然感染した2匹の大人のサルの血液を輸血した。レシピエントアニマルのウイルス感染並びに持続、抗体反応及び臨床的変化を観察した。接種1年後の結果は、SFVが全血によって感染する場合があることを示した。これらの事実は、血液ドナーについてのスクリーニングが必要なことを暗示させるものであった。

使用上の注意記載状況・その他参考事項等

代表としてテタノブリンーIHの記載を示す。

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高力価の破傷風抗毒素を含有する血漿を原料として、Cohnの低温エタノール分画で得た画分からポリエチレングリコール4000処理、DEAEセファデックス処理等により抗破傷風人免疫グロブリンを濃縮・精製した製剤であり、ウイルス不活化・除去を目的として、製造工程において60℃、10時間の液状加熱処理及び濾過膜処理（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

SFVが輸血により伝播する可能性を示唆する報告である。
血漿分画製剤からのSFV伝播の事例は報告されていない。また、万一原料血漿にSFVが混入したとしても、HIV-1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。

今後の対応

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。

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the island state of Singapore. Additionally, in 2003–2004, we screened 140 whole blood samples from newly quarantined *M. nemestrina* from Indonesia. Of the 37 Singaporean *M. fascicularis*, an overall seroprevalence of 86.5% (32/37) was found in five different troops and 70% (26/37) were found to contain detectable levels of SRV DNA. Among the 140 Indonesian *M. nemestrina*, the seroprevalence was 12% (17/140) and 8% (11/140) were PCR positive. Nine of the Indonesian *M. nemestrina* were identified as PCR positive when using primers targeting a conserved region of the envelope transmembrane region (gp20) but only two animals were positive when using primers to a more variable region of the envelope outer membrane (gp70). All Singapore PCR positive animals reacted to both gp20 and gp70 primers. Upon amplification and sequencing of the SRV envelope gene from the positive Indonesian *M. nemestrina*, all contained a new SRV isolate that is only 70–78% related to the other SRV serotypes one through six. The new SRV can be propagated on Raji cells but does not produce severe CPE. SRV-2 Western blots of the antibody positive Indonesian *M. nemestrina* appear weaker and less specific for the gp70 outer membrane glycoprotein than antibody positive *M. fascicularis* from Singapore. Phylogenetic analysis indicates that the Singaporean *M. fascicularis* SRV isolates are very closely related to other reported SRV-2 isolates (95–98% identity) while the new *M. nemestrina* Indonesian isolates are unique (70–80% identity) and may represent a new serotype. As we and others have not been able to isolate SRV-2 from other macaque species in wild settings, and SRV-2 is clearly prevalent in wild *M. fascicularis* and is considerably different than SRV in wild *M. nemestrina*, it is possible that *M. fascicularis* is the natural reservoir for SRV-2 and cross-species transmissions from *M. fascicularis* to other species may be responsible for the isolation of SRV-2 during outbreaks at primate centers.

ABSTRACT #56

CROSS-SPECIES TRANSMISSION OF SIMIAN FOAMY VIRUS FROM FERAL ASIAN MACAQUES TO HUMANS

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Feral populations of Asian macaques come in frequent contact with humans in settings ideal for transmission of simian retroviruses. Simian foamy viruses (SFV) are considered non-pathogenic infections of non-human primates (NHP) and recent studies have shown infrequent transmission to humans at primate research facilities, zoos, and in bushmeat hunters in Africa. No data have been published concerning cross-species infection of humans by Asian macaques. The significance of SFV infection in humans is unknown, however, studies to date have not identified any disease associated with long-term infection. In this study of 81 humans with frequent contact with macaques at Buddhist Temples in Bali, Indonesia, we identified one person with antibodies to SFV. Nested PCR assessment of whole blood from this individual confirmed infection. PCR products were cloned and sequenced and compared with SFVs from monkeys in the same geographic region as well as other Macaca species. Alignment and phylogenetic analysis of SFVhu-BH66 with Asian monkey SFVs indicated that SFVhu-BH66 was most closely related to an SFV-infected monkey from the area. The human origin of the BH66 blood sample was confirmed by PCR cloning, sequencing of the 12S RNA subunit of mitochondrial DNA, and phylogenetic analysis. These data further illustrate the potential for simian retroviral transmission to humans in enzootic areas and point to the risk for development of a new emerging infectious disease.

ABSTRACT #57

STUDIES OF SIMIAN FOAMY VIRUS TRANSMISSION BY BLOOD

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Simian foamy viruses (SFVs) are widespread in various non-human primate species. Although the mode of transmission has not been well studied, the high prevalence is thought to be due to transmission via saliva. Cross-species infection in humans has been found in Central African hunters, and can occur due to accidental, occupational exposure to infected animals. The infection results in long-term virus persistence. In contrast to the infection in its natural host, there has been no evidence of SFV human-to-human transmission. However, due to the AIDS epidemic, it is cautionary to prevent retroviral zoonosis, even in the absence of disease in its natural host. Additionally, it is imperative to avoid further virus adaptation by human passage. Recently, due to reports of SFV infections in humans, there has been a concern regarding potential SFV transmission by blood donors. To investigate whether SFV can be transmitted by blood, we transfused SFV-negative rhesus macaques with blood from two adult macaque donors that were naturally infected with biologically and genetically distinct SFVs. The recipient animals were monitored for virus infection and persistence, humoral antibody response and clinical changes. The results at 1-year post-inoculation indicate that SFV can be transmitted by whole blood, in some cases. These findings could have implications for blood donor screening.

ABSTRACT #58

REAL-TIME TAQMAN PCR AS A TOOL FOR SIMIAN RETROVIRAL DIAGNOSTICS

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Retroviral diagnostic testing is a critical tool for accurately assessing infection status of non-human primates (NHP). Determining simian retroviral infection presents unique challenges associated with the individual characteristics of each virus. We developed quantitative qTaqMan PCR to screen Asian and African NHPs for SRV, SIV, and STLV, the major exogenous retroviruses found in Old World Primates. Sensitivity and specificity correlated well with nested PCR in most instances. For SRV testing, comparison of antibody-based Western blotting with TaqMan PCR suggests that both assays are necessary to conclusively identify positive animals. Strain-specific SRV TaqMan assays were required to eliminate false positives due to endogenous SRV of macaques. SRV-1, -3, and SRV-2 assays have been developed with sensitivities of < 10 DNA copies/10^5 lymphocytes. Viral loads were examined for 54 SRV-2+ cynomolgus macaques by TaqMan DNA PCR. Copy numbers varied from < 10 copies to as high as 1.4×10^6 copies/10^5 cells with a median of 22 DNA copies. Similar sensitivities were found for to screen blood samples for retroviral infections with the added advantage in minimizing false positive sampling commonly seen with nested PCR.

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		1	報告日	第一報入手日 2006 年 5 月 30 日	新医薬品等の区分	厚生労働省処理欄
一 般 的 名 称	別紙のとおり		研究報告の公表状況	Public Health Agency of Canada Web site, Travel Health Advisories, May 26, 2006	公表国 カナダ	
販 売 名（企 業 名）	別紙のとおり					

研究報告の概要

（問題点：致死率が低い、蚊によって媒介される伝染病「チクングンヤ熱」が、インド洋南西地域の島、インドで蔓延し、最近では死亡者も増えている。）

インド洋南西地域の島では、2005 年 3 月～2006 年 4 月 22 日にチクングンヤ感染症例が合計 3,877 例確認された。確認されていない感染者数が多く存在し、実際の症例数は 255,000 例近い可能性があると推定されている。

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ており、2006 年 4 月 20 日時点で、153,324 例が確定例および可能性例として報告されている

チクングンヤウィルス（Chikungunya）は、ヒトスジシマカ（Aedes albopictus）（aegypti）などにより媒介されるトガウィルス科（Togaviridae）のアルファウィルス（Alphavirus）を病原とする。トガウィルス科は 1 本鎖の RNA ウィルスで、同類にはデング熱、日本脳炎、ウェストナイルウィルス、黄熱などの病原であるフラビウィルス（flavivirus）（フラビウィルス科）がある。

チクングンヤウィルス感染の症状
ウィルスに感染しても軽症で済む人が多い。チクングンヤウィルスは潜伏期間 3－5 日、発症すると激しい発疹、衰弱するほどの強度な関節の痛み、脱水症状を呈する。ワクチンは開発されていない。

使用上の注意記載状況・その他参考事項等

記載なし

報告企業の意見	今後の対応
別紙のとおり	現時点においては、特段の対応は不要と考えるが、今後とも関連情報の収集に努め、本剤の安全性の確保を図っていきたい。

別紙

一般的名称	①人血清アルブミン②人血清アルブミン③人血清アルブミン④人免役グロブリン⑤乾燥ペプシン処理人免疫グロブリン⑥乾燥スルホ化人免疫グロブリン⑦乾燥スルホ化人免疫グロブリン⑧乾燥濃縮人活性化プロテインC⑨乾燥濃縮人血液凝固第Ⅷ因子⑩乾燥濃縮人血液凝固第Ⅸ因子⑪乾燥抗破傷風人免疫グロブリン⑫抗ＨＢｓ人免疫グロブリン⑬トロンビン⑭フィブリノゲン加第ＸⅢ因子⑮乾燥濃縮人アンチトロンビンⅢ⑯ヒスタミン加人免疫グロブリン製剤⑰人血清アルブミン⑱人血清アルブミン⑲乾燥ペプシン処理人免役グロブリン⑳乾燥人血液凝固第Ⅸ因子複合体㉑沈降精製百日せきジフテリア破傷風混合ワクチン㉒沈降精製百日せきジフテリア破傷風混合ワクチン㉓沈降精製百日せきワクチン㉔乾燥弱毒生風しんワクチン㉕乾燥弱毒生おたふくかぜワクチン
販売名（企業名）	①献血アルブミン20“化血研”②献血アルブミン25“化血研”③人血清アルブミン“化血研”④“化血研”ガンマーグロブリン⑤献血静注グロブリン“化血研”⑥献血ベニロンーＩ⑦ベニロン⑧注射用アナクトC2,500単位⑨コンファクトＦ⑩ノバクトM⑪テタノセーラ⑫ヘパトセーラ⑬トロンビン“化血研”⑭ボルヒール⑮アンスロビンＰ⑯ヒスタグロビン⑰アルブミン20%化血研⑱アルブミン5%化血研⑲静注グロブリン⑳ノバクトＦ㉑DPT“化血研”シリンジ㉒沈降精製百日せきジフテリア破傷風混合ワクチン㉓沈降精製百日せきワクチン㉔乾燥弱毒生風しんワクチン㉕乾燥弱毒生おたふくかぜワクチン「化血研」㉖アンスロビンＰ1500注射用
報告企業の意見	弊所の血漿分画製剤に対するウイルス安全性は、原料血漿におけるNAT及び血清学的検査によるスクリーニング、製造工程での効果的なウイルス不活化・除去、更には小分品でのNAT、血清学的検査による確認というステップにより確保されている。製造工程のウイルス除去・不活化能は、「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について（医薬発第1047号）」に従い、原料に混入する可能性のあるウイルスを考慮したモデルウイルスを選定し、ウイルスプロセスバリデーションを実施し評価を行っている。 チクングンヤウイルスは、トガウイルス科に属しており、弊所におけるトガウイルスに対する製造工程の安全性評価は、モデルウイルスとしてBVDV（ウシウイルス性下痢ウイルス）を用いてウイルスプロセスバリデーションを実施している。弊所の血漿分画製剤は、その製造工程中にウイルス安全対策工程として「アフィニティークロマト工程」「ウイルス除去膜工程」や「加熱工程」等が導入されている。この原理が異なるウイルス安全対策工程については、上記ウイルスプロセスバリデーションの結果より、チクングンヤウイルスに対する不活化、除去効果が確認されている。また、この様に、ウイルスプロセスバリデーションにより検証されたウイルス除去・不活化工程を経た弊所の血漿分画製剤において、その臨床使用上もチクングンヤウイルスの感染報告例はない。 また、ヒト血漿由来のアポセルロプラスミンを沈降精製百日せきジフテリア破傷風混合ワクチンの製造工程において使用しているが、以下の理由により安全と考えられる。 アポセルロプラスミンの製造には、ウイルス除去膜が用いられており、チクングンヤウイルスは同工程において効果的に除去されると考えられる。更に、アポセルロプラスミンは、65℃で18時間加熱工程を行っている。仮に原料自体にチクングンヤウイルスが混入していたとしても、製造過程で充分にウイルスの不活化・除去はできているものと考えられる。 弊所製品のチクングンヤウイルスに対する安全性は高いレベルで保たれていると考えるが、今後とも情報収集に努め、更なる安全性の向上を図っていきたい。

INF2006-004

TRAVEL HEALTH ADVISORY

Outbreak of Chikungunya Virus: South West Indian Ocean and India

Updated: May 26, 2006

The Public Health Agency of Canada (PHAC) continues to monitor outbreaks of chikungunya virus - a mosquito-borne disease - on islands in the south western region of the Indian Ocean and in India.

PHAC was recently notified of illness caused by chikungunya infection in four Canadian tourists. The four Canadians traveled to Reunion Island, Mauritius, Seychelles, and/or the Indian coast. Symptom onset occurred between February to early March. Several European countries have also reported imported cases in people returning from these islands: France (160 imported cases), Germany, Italy, Norway and Switzerland.

Islands in the South West Indian Ocean

Between March 2005 and April 22, 2006 a total of 3877 confirmed cases of chikungunya infection have been officially reported through Réunion's surveillance network. Health officials estimate that a significant number of infections remain undetected and that the actual number of cases during this period may number close to 255 000.

Chikungunya virus activity has also been reported on other islands in the south west Indian Ocean, including Mayotte, Mauritius, and the Seychelles.

Several European countries have reported imported cases in people returning from these islands: France (160 imported cases), Germany, Italy, Norway and Switzerland.

India

Since December 2005, outbreaks of chikungunya virus have been reported in the states of Karnataka, Maharastra, and Andra Pradesh. As of April 20, 2006 153 324 confirmed and probable

cases have been reported.

Measures are being undertaken to control these outbreaks. Intensive measures to interrupt transmission, including increased surveillance and mosquito-control measures, continue to be implemented by local authorities.

Source: Eurosurveillance, Institut de veille sanitaire (France), World Health Organization

Chikungunya virus is most commonly transmitted to humans through the bite of an infected mosquito, specifically mosquitoes of the Aedes genus, which usually bite during daylight hours.

Symptoms of infection, which generally last three to seven days, include the sudden onset of fever, chills, headache, nausea, vomiting, severe joint pain (arthralgias), and rash. Although rare, the infection can result in meningoencephalitis (swelling of the brain), especially in newborns and those with pre-existing medical conditions. Pregnant women can pass the virus to their fetus . Residual arthritis, with morning stiffness, swelling, and pain on movement, may persist for weeks or months after recovery. Severe cases of chikungunya can occur in the elderly, in the very young (newborns), and in those who are immunocompromised. C hikungunya outbreaks typically result in several hundreds or thousands of cases but deaths are rarely encountered.

Chikungunya virus is most likely of African origin. Recent outbreaks have occurred in Sub-Saharan Africa, India, South-east Asia, and the Philippines.

There is no vaccine that protects against chikungunya virus. Treatment for chikungunya typically involves treating the symptoms and includes bed-rest and the use of non-aspirin analgesics during the phase of illness where the symptoms are most severe. Using protective measures to prevent being bitten by an infected mosquito remains the only means to reduce the risk of exposure.

Recommendations

The Public Health Agency of Canada reminds travellers to tropical and subtropical areas of the world that they may be at risk for contracting mosquito-borne diseases, such as malaria, dengue, Japanese encephalitis, yellow fever, and other less common diseases like chikungunya. Travellers are strongly encouraged to consult their personal physician or a travel medicine practitioner to discuss their individual risk of exposure to such diseases.

Personal Measures to Avoid Mosquitoes

The Public Health Agency of Canada strongly recommends that travellers take the following personal precautions to reduce the risk of exposure to mosquitoes:

- remain in well-screened or completely enclosed, air-conditioned areas;

- wear light-coloured clothing with full-length pant legs and sleeves; and
- use insect repellent on exposed skin.

The use of insect repellent on exposed skin is strongly recommended. Of the insect repellents registered in Canada, those containing 'N, N diethyl-m-toluamide' (DEET) are the most effective. **There are specific things you should know about DEET, especially regarding its use on young children.**

- Use DEET-based products as repellents on exposed skin. The higher the concentration of DEET in the repellent formulation, the longer the duration of protection. However, this relation reaches a plateau at about 30% to 35%. DEET formulations that are "extended duration" (ED), such as polymers, are generally considered to provide longer protection times, and may be associated with less DEET absorption. Formulations over 30% are not currently available in Canada, although they are available internationally, including in the United States. It should be noted, however, that products sold outside Canada have not been evaluated by Health Canada. Most repellents containing "natural" products are effective for shorter durations than DEET and for this reason are not considered the preferred products for protecting against mosquito bites.
- Regulatory agencies in western nations may differ regarding the recommended maximum concentration and application rates of DEET, especially for children. The Committee to Advise on Tropical Medicine and Travel (CATMAT) is satisfied that, for travel outside of Canada where the risk of malaria outweighs the risk of any important adverse reaction to DEET, the threshold for use of DEET should be low.
- CATMAT recommends that concentrations of DEET up to 35% can be used by any age group.
- **For children**, alternative personal protective measures, such as mosquito nets treated with insecticide, should be the first line of defense, especially for infants less than 6 months of age. Portable mosquito nets, including self-standing nets, placed over a car seat, a crib, playpen, or stroller help protect against mosquitoes. However, as a complement to the other methods of protection, the judicious use of DEET should be considered for children of any age. Recent medical literature from Canada suggests that DEET does not pose a significant or substantial extra risk to infants and children.
- DEET/sunscreen combination products are not generally recommended, because DEET can decrease the efficacy of sunscreens. As well, sunscreens should be used liberally and often while DEET should be used sparingly and only as often as required. If application of both is necessary, the Canadian Dermatology Association recommends that the sunscreen be applied first and allowed to penetrate the skin for 20 minutes, prior to applying DEET.

The Public Health Agency of Canada's Committee to Advise on Tropical Medicine and Travel (CATMAT) produces evidence based statements and guidelines. **For additional information on Arthropod Bite Prevention** visit CATMAT's Statement on

Personal Protective Measures to Prevent Arthropod Bites.

As a reminder...

The Public Health Agency of Canada routinely recommends that Canadian international travellers consult their personal physician or a **travel clinic four to six weeks** prior to international travel, regardless of destination, for an individual risk assessment to determine their individual health risks and their need for vaccination, preventative medication, and personal protective measures.

The Public Health Agency of Canada recommends, as well, that travellers who become sick or feel unwell on their return to Canada should seek a medical assessment with their personal physician. Travellers should inform their physician, without being asked, that they have been travelling or living outside of Canada, and where they have been.

Additional information from the Public Health Agency of Canada:

- More information about **arthropod bite prevention**;
- More information about ***Canadian recommendations for the prevention and treatment of malaria among international travellers***;
- More information about **dengue**;
- For information about **yellow fever**.

Last Updated: 2006-05-26 ▲ Important Notices

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		1	報告日	第一報入手日 2006 年 5 月 12 日	新医薬品等の区分	厚生労働省処理欄
一 般 的 名 称	別紙のとおり		研究報告の公表状況	小児の下気道感染症患者からのヒトボカウイルス（Human Bocavirus）の検出 第80回 日本感染症学会総会・学術講演会	公表国 日本	
販 売 名（企 業 名）	別紙のとおり					

研究報告の概要

（問題点：広く世界中に分布して、さまざまな呼吸器感染症の原因ウイルスである HboV が、日本国内の小児からも検出された。）

2005 年、スウェーデンの小児の鼻咽頭スワブから、新しいヒト呼吸器感染症ウイルス（ヒトボカウイルス HBoV と仮命名）が同定された。このウイルスは、パルボウイルスかパルボウイルス亜科ボカウイルス属に属し、小児の気道感染症の原因の一つと推定されている。そこで、日本国内の HboV 検出状況を調査した。

2002 年 10 月から 2003 年 9 月、2005 年 1 月から 7 月の 2 シーズンにわたり、318 人（平均年齢 21.3 ヶ月、男女比 1.4：1）の小児下気道感染症患者から採取した鼻咽頭スワブより抽出した DNA を鋳型として PCR で検出した。

18／318（5.7%）の検体から HboV が検出された。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 の塩基配列はよく保たれていた。診断病名は肺炎（6 名）、喘息様気管支炎（6 名）、気管支炎（2 名）、細気管支炎（2 名）、気管支喘息（1 名）、咽頭気管支炎（1 名）で、16 名が入院を必要とした。全症例に咳、発熱を認め、8 名の患者の X 線写真に所見を認めた。

日本国内の小児からも HBoV が検出された。HBoV は広く世界中に分布して、様々な呼吸器感染症の原因ウイルスになっていると推察された。

使用上の注意記載状況・その他参考事項等

記載なし

報告企業の意見	今後の対応
別紙のとおり	現時点においては、特段の対応は不要と考えるが、今後とも関連情報の収集に努め、本剤の安全性の確保を図っていきたい。

別紙

一般的名称	①人血清アルブミン②人血清アルブミン③人血清アルブミン④人免役グロブリン⑤乾燥ペプシン処理人免疫グロブリン⑥乾燥スルホ化人免疫グロブリン⑦乾燥スルホ化人免疫グロブリン⑧乾燥濃縮人活性化プロテインC⑨乾燥濃縮人血液凝固第Ⅷ因子⑩乾燥濃縮人血液凝固第Ⅸ因子⑪乾燥抗破傷風人免疫グロブリン⑫抗HBs人免疫グロブリン⑬トロンビン⑭フィブリノゲン加第XIII因子⑮乾燥濃縮人アンチトロンビンIII⑯ヒスタミン加人免疫グロブリン製剤⑰人血清アルブミン⑱人血清アルブミン⑲乾燥ペプシン処理人免役グロブリン⑳乾燥人血液凝固第Ⅸ因子複合体㉑沈降精製百日せきジフテリア破傷風混合ワクチン㉒沈降精製百日せきジフテリア破傷風混合ワクチン㉓沈降精製百日せきワクチン㉔乾燥弱毒生風しんワクチン㉕乾燥弱毒生おたふくかぜワクチン
販売名(企業名)	①献血アルブミン20“化血研”②献血アルブミン25“化血研”③人血清アルブミン“化血研”④“化血研”ガンマーグロブリン⑤献血静注グロブリン“化血研”⑥献血ベニロンーⅠ⑦ベニロン⑧注射用アナクトC2,500単位⑨コンファクトF⑩ノバクトM⑪テタノセーラ⑫ヘパトセーラ⑬トロンビン“化血研”⑭ボルヒール⑮アンスロビンP⑯ヒスタグロビン⑰アルブミン20%化血研⑱アルブミン5%化血研⑲静注グロブリン⑳ノバクトF㉑DPT“化血研”シリンジ㉒沈降精製百日せきジフテリア破傷風混合ワクチン㉓沈降精製百日せきワクチン㉔乾燥弱毒生風しんワクチン㉕乾燥弱毒生おたふくかぜワクチン「化血研」
報告企業の意見	弊所の血漿分画製剤に対するウイルス安全性は、原料血漿におけるNAT及び血清学的検査によるスクリーニング、製造工程での効果的なウイルス不活化・除去、更には小分品でのNAT、血清学的検査による確認というステップにより確保されている。製造工程のウイルス除去・不活化能は、「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について（医薬発第1047号）」に従い、原料に混入する可能性のあるウイルスを考慮したモデルウイルスを選定し、ウイルスプロセスバリデーションを実施し評価を行っている。 ヒトボカウイルスHBoVは、パルボウイルス科パルボウイルス亜科ボカウイルス属に属しており，弊所におけるパルボウイルスに対する製造工程の安全性評価は、モデルウイルスとしてPPV（ブタパルボウイルス）を用いてウイルスプロセスバリデーションを実施している。弊所の血漿分画製剤は、その製造工程中にウイルス安全対策工程として「アルコール分画工程」「ウイルス除去膜工程」や「加熱工程」等が導入されている。この原理が異なるウイルス安全対策工程については、上記ウイルスプロセスバリデーションの結果より、パルボウイルスに対する不活化、除去効果が確認されている。また、この様に、ウイルスプロセスバリデーションにより検証されたウイルス除去・不活化工程を経た弊所の血漿分画製剤において、その臨床使用上もパルボウイルスの感染報告例はない。 また、ヒト血漿由来のアポセルロプラスミンを沈降精製百日せきジフテリア破傷風混合ワクチンの製造工程において使用しているが、以下の理由により安全と考えられる。 アポセルロプラスミンの製造には、ウイルス除去膜が用いられており、パルボウイルスを同工程において効果的に除去されると考えられる。更に、アポセルロプラスミンは、65℃で18時間加熱工程を行っている。 これらのことから、仮に原料自体にパルボウイルスが混入していたとしても、製造過程で充分にウイルスの不活化・除去はできているものと考えられる。 弊所製品のパルボウイルスに対する安全性は高いレベルで保たれていると考えるが、今後とも情報収集に努め、更なる安全性の向上を図っていきたい。

X0660021

172　小児の下気道感染症患者からのヒトボカウイルス（Human Bocavirus）の検出

北海道大学大学院医学研究科病態制御学専攻生殖・発達医学講座小児科学分野[1]，
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○石黒信久[1,3]，遠藤理香[1]，石古博昭[3]，菊田英明[1,4]

【目的】2005年，スウェーデンの小児の鼻咽頭スワブから，新しいヒト呼吸器感染症ウイルスが同定された（PNAS 2005 Sep 6；102（36）：12891-6）．ヒトボカウイルスHuman bocavirus（HBoV）と仮命名されたこのウイルスは，パルボウイルス科パルボウイルス亜科ボカウイルス属に属し，小児の気道感染症の原因の一つと推定されている．そこで，日本国内のHBoV検出状況を調査することを本研究の目的とした．
【方法】2002年10月から2003年9月，2005年1月から7月の2シーズンにわたり，318人（平均月齢21.3か月，男女比1.4：1）の小児下気道感染症患者から採取した鼻咽頭スワブより抽出したDNAを鋳型とし，5'-GAGCTCTGTAAGTACTATTAC-3'，5'-CTCTGTGTTGACTGAATACAG-3'をプライマーとしてPCR(94℃9分に続き，94℃1分，54℃1分，72℃2分，35サイクル）を行った．PCR産物を1.5%アガロースゲルに泳動するとともに，塩基配列を決定した．RSV，インフルエンザA&B，hMPV陽性者は対象から除外した．
【結果】18/318（5.7%）の検体からHBoVが検出された．HBoVが検出された患者の年齢は7か月～3歳で，検出月は1月から5月に集中していた．HBoVの塩基配列は良く保たれていた．診断病名は肺炎（6名），喘息様気管支炎（6名），気管支炎（2名），細気管支炎（2名），気管支喘息（1名），喉頭気管気管支炎（1名）で，16名が入院を必要とした．全症例に咳嗽と発熱を認め，最高体温は37.5-40℃，37.5℃以上の発熱持続期間は1-8日であった．8名の患者の胸部X線写真に所見を認めた．
【考案】日本国内の小児からもHBoVが検出された．HBoVは広く世界中に分布して，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．
（非学会共同研究者：馬暁明，海老原敬）

第80回日本感染症学会総会学術講演会(2006.4.20,21)
感染症学雑誌(80,S248,2006.3)

別紙様式第 2-1

医薬品　研究報告　調査報告書

文献ＩＤ：Baxter2006-001

報告番号・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	乾燥イオン交換樹脂処理人免疫グロブリン		研究報告の公表状況	鳥インフルエンザウイルスの血液感染の可能性について、Emerging Infections Diseases/WWW.cdc.gov/eid/Vol.12, No.6, June 2006 に掲載があった。	公　表　国	
販売名（企業名）	ガンマガード（バクスター株式会社）					

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
問題点（血液感染の可能性に関連した鳥インフルエンザウイルスの伝播） 研究報告の題目：H5N1 インフルエンザＡウイルスと感染人血漿　タイ、2006 年 2005 年 12 月にタイにおいて鳥インフルエンザウイルス（H5N1）に感染し、死亡した症例の血漿から鳥インフルエンザウイルス（H5N1）が分離された。本ウイルスは遺伝子解析の結果、A/Thailand/NK165/05 accession no. DQ 372591-8 株として同定され、発育鶏卵培養により増殖能も確認された。 報告者は、患者の血漿から増殖能のあるウイルスを検出したことから、人の血液を介しての伝播についての可能性を提起し、H5N1 鳥インフルエンザに感染していると疑われる症例の血清あるいは血漿サンプルを処理して輸送する場合の取り扱いについて、注意すべきと報告している。 詳細は添付文献のとおり。	２．重要な基本的注意 (1) 本剤の原材料となる血漿については、FDA で認可された方法で HBs 抗原、抗 HCV 抗体、抗 HIV-1 及び HIV-2 抗体陰性であることを確認し、かつ ALT（GPT）値でスクリーニングを実施している。さらに、プールした試験血漿については、HBV-DNA、HCV-RNA、HIV-1-RNA、HIV-2-RNA 及び HAV-RNA 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。同様に、ヒトパルボウイルス B19-DNA についてはプールした試験血漿で核酸増幅検査（NAT）を実施し、10^5IU/mL 以下であることを確認した健康人血漿を用いている。本剤は、Cohn の低温エタノール分画法によって得られた免疫グロブリン画分を、TNBP／TritonX-100／Tween80 処理することによりエンベロープを有するウイルスを不活化し、さらにイオン交換樹脂処理により夾雑たん白やウイルスを排除する工程を施しているが、ウイルス等の感染

報告企業の意見	今後の対応
本剤の製造工程におけるウイルス不活化／除去工程において、当該ウイルスと同科ではないが、同じ RNA ウイルスであるフラビウイルス科の C 型肝炎ウイルス（HCV）の不活化・除去バリデーション試験に用いられるモデルウイルスであるウシ下痢性ウイルス（BVDV）は、不活化・除去されることが示されていることより、輸血に関連した血漿分画製剤による感染の可能性は極めて小さいと考える。	当該感染症に関し、引き続き、情報の収集を行っていく。 また、同様に同一生物種等から人に感染すると認められる疾病に関する情報の収集に努める

		性を完全には否定できないので、投与に際しては、次の点に十分注意すること。 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。 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。

感染症の用語は、MedDRA/J version (9.0) を使用。

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H5N1 Influenza A Virus and Infected Human Plasma

To the Editor: Since January 2004, a total of 22 persons have been confirmed infected with avian influenza A virus (H5N1) in Thailand; 14 of these patients died. Three waves of outbreaks occurred during the past 2 years. The last patient of the third wave was a 5-year-old boy whose symptoms developed on November 28, 2005; he was hospitalized on December 5 and died 2 days later. The child resided in the Ongkharak District, Nakhon Nayok Province, 70 km northeast of Bangkok. Villagers informed the Department of Livestock after the patient's illness was diagnosed. Five dead chickens had been reported in this area from November 28 to December 1, 2005. Samples from these chickens could not be obtained, thus, no H5N1 testing was performed. The boy had fever, headache, and productive cough for 7 days before he was admitted to the Her Royal Highness Princess Maha Chakri Sirindhorn Medical Center. Clinical examination and chest radiograph showed evidence of lobar pneumonia. He was treated with antimicrobial drugs (midecamycin and penicillin G) and supportive care, including oxygen therapy. On December 7, the patient's condition worsened, and severe pneumonia with adult respiratory distress syndrome developed. Laboratory tests showed leukopenia (2,300 cells/mm^3), acidosis, and low blood oxygen saturation by cutaneous pulse oximetry (81.6%). Oseltamivir was administered after his parents informed hospital staff about the boy's contact with the dead chicken. However, the boy died the same day; no autopsy was performed. On December 9, the cause of death was declared by the Ministry of Public Health to be H5N1 influenza virus.

A blood sample was collected from the patient on December 7; anticoagulation was accomplished with ethylenediaminetetraacetic acid (EDTA) for repeated biochemistry analysis and complete blood count. The plasma from the EDTA blood sample was separated 2 days later and stored at –20°C for 12 days. The sample was subsequently given to the Center of Excellence in Viral Hepatitis, Faculty of Medicine, Chulalongkorn University, for molecular diagnosis and then stored at –70°C, where specific precautions implemented for handling highly infectious disease specimens such as H5N1 influenza virus were observed. Plasma was examined by multiplex reverse transcription–polymerase chain reaction (RT-PCR) (*1*) and multiplex real-time RT-PCR (*2*), both of which showed positive results for H5N1 virus. The virus titer obtained from the plasma was 3.08×10^3 copies/mL. The plasma specimen was processed for virus isolation by embryonated egg injection, according to the standard protocol described by Harmon (*3*). Briefly, 100 µL 1:2 diluted plasma was injected into the allantoic cavity of a 9-day-old embryonated egg and incubated at 37°C. The infected embryo died within 48 hours, and the allantoic fluid was shown to contain 2,048 hemagglutinin (HA) units; also, subtype H5N1 was confirmed (*1,2*). Whole genome sequencing was performed and submitted to the GenBank database under the strain A/Thailand/NK165/05 accession no. DQ 372591-8. The phylogenetic trees of the HA and neuraminidase (NA) genes were constructed by using MEGA 3 (*4*) for comparison with H5N1 viruses isolated from humans, tigers, and chickens from previous outbreaks in 2004 and 2005 (Figure). The sequence analyses of the viruses showed that the HA cleavage site contained SPQRERRKKR, which differed from the 2004 H5N1 virus by an arginine-to-lysine substitution at posi-

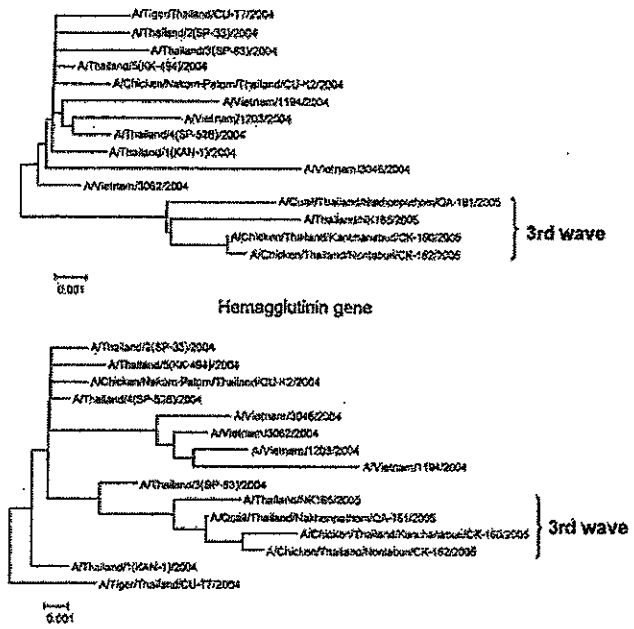

Figure. Phylogenetic analysis of the hemagglutinin and neuraminidase genes of H5N1 from study patient compared with sequences from previous outbreaks (2004–2005).

tion 341. That finding had also been observed in wild bird species during earlier outbreaks in Thailand in 2004 (*5*). Similar to the 2004–2005 H5N1 isolates from Thailand, a 20–amino acid deletion at the NA stalk region was observed. Moreover, the amino acid residues (E119, H274, R292, and N294) of the NA active site were conserved, which suggests that the virus was sensitive to oseltamivir. In addition, a single amino acid substitution from glutamic acid to lysine at position 627 of PB2 showed increased virus replication efficiency in mammals (*6*).

Observing live influenza virus in human serum or plasma is unusual. However, in 1963, low quantities of virus were isolated from blood of a patient on day 4 of illness (*7*), and in 1970, the virus was cultivated from blood specimens from 2 patients (*8*). Recently, a fatal case of avian influenza A (H5N1) in a Vietnamese child was reported. The diagnosis was determined by isolating the virus from cerebrospinal fluid, fecal, throat, and serum specimens (*9*); viral RNA was found in 6 of 7 serum specimens 4–9 days after the onset of illness (*10*). In this case, the H5N1 virus could be isolated from plasma on day 10 after symptoms developed. This case showed the virus in the patient's blood, which raises concern about transmission among humans. Because probable H5N1 avian influenza transmission among humans has been reported (*11*), this case should be a reminder of the necessity to carefully handle and transport serum or plasma samples suspected to be infected with H5N1 avian influenza. Because viable virus has been detected in blood samples, handling, transportation, and testing of blood samples should be performed in a biosafety (category III) containment laboratory to prevent the spread of the virus to healthcare and laboratory workers.

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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	①ポリエチレングリコール処理抗破傷風人免疫グロブリン ②乾燥抗破傷風人免疫グロブリン	研究報告の公表状況	BMJ Online doi:10.1136/bmj.38804.511644.55	公表国 イギリス		
販売名（企業名）	①テタノブリンーIH（ベネシス） ②テタノブリン（ベネシス）					

研究報告の概要

＜目的＞
プリオン陽性と判定された虫垂検体から抽出されたDNAのプリオン蛋白遺伝子コドン129の分析
＜調査対象の検体＞
英国のvCJD発生調査の一つとして回顧的にプリオンについて試験を実施した12,674の虫垂及び扁桃の検体から陽性と判定された3つの虫垂検体。これら検体が得られた患者の年齢は外科手術時点で20-29歳であり、これら手術が行われたのは1996-9年である。
＜結果＞
3検体の内2検体は十分な量のDNAが利用可能であり、両方共がプリオン蛋白遺伝子コドン129はバリンのホモ接合体であった。
＜結論＞
これが、プリオン蛋白におけるコドン129のバリンのホモ接合体がvCJDに対する感受性が強い証拠を初めて示したものである。試験が行われたvCJDの臨床例はこれまで全て、メチオニンのホモ接合体に起こっている。そして、医原性vCJDであることがほぼ間違いない単一例が一人の患者に見つかっているが、この患者はこの遺伝子座にメチオニン／バリンのヘテロ接合体を有していた。バリンのホモ接合体のコドン129プリオン蛋白遺伝子を有するvCJD感染者は長い潜伏期間を有している可能性があり、この間に水平感染が血液ドナー又は無症候期におけるこれらの感染者に使用された汚染手術用具のいずれかから起きる可能性がある。

使用上の注意記載状況・その他参考事項等

代表としてテタノブリンー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。

報告企業の意見	今後の対応
プリオンについて試験を実施した12,674の虫垂及び扁桃の検体から陽性と判定された3つの虫垂検体のうち2つの虫垂検体のプリオン蛋白遺伝子コドン129がバリンのホモ体であったことから、バリンのホモ接合体のコドン129プリオン蛋白遺伝子を有するvCJD感染者は長い潜伏期間を有している可能性があり、この潜伏期間中に血液ドナー又は無症候期におけるこれらの感染者に使用された汚染手術用具のいずれかから水平感染が起きる可能性があることを示唆する報告である。 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。	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。

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BENE2006-012

Research

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 McCardle, David A Hilton

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 3 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 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 appendixes, 1565 tonsils) removed between 1995 and 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 number of patients diagnosed with vCJD in 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 (MM, MV, VV).

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.

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 methods identical to those used in this study, though the spleen and lymph nodes gave positive results.[5] *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 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. JWI is guarantor.

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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.

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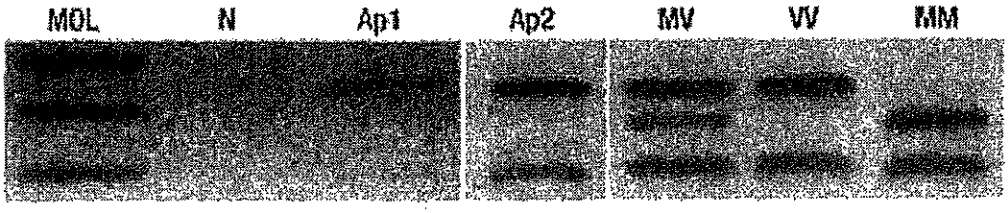

Restriction digest pattern for *PRNP* codon 129 genotype analysis in two paraffin section tissue samples (shown combined). The test sample results clearly show banding patterns equivalent to the VV genotype control (Mol=molecular weight ladder, N=PCR negative control, Ap1=appendix tissue from positive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

What is already known on this topic

A recent prevalence study of accumulation of prion protein (as a marker for variant Creutzfeldt-Jakob disease) in appendix and tonsil specimens in the UK found three cases in12 674 samples, 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 they were valine homozygotes at codon 129 in the prion protein gene, indicating that this genetic subgroup (which is a different subgroup to that in which all cases of vCJD so far have 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

別紙様式第2-1

No. 18

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. 3. 25	該当なし	

一般的名称	解凍人赤血球濃厚液	研究報告の公表状況	Yamada M; Variant CJD Working Group, Creutzfeldt-Jakob Disease Surveillance Committee, Japan. Lancet. 2006 Mar 11;367(9513):874.	公表国 日本
販売名(企業名)	解凍赤血球濃厚液「日赤」(日本赤十字社) 照射解凍赤血球濃厚液「日赤」(日本赤十字社)			

研究報告の概要

○変異型クロイツフェルト・ヤコブ病(vCJD)日本初の症例は周期性脳波を示していた

2004年2月、50歳の日本人男性の症例がCJDサーベイランス委員会に報告された。患者は1990年代前半に英国に24日間、フランスに3日間、vCJDの報告がない欧州の他の国に2週間滞在していた。手術歴や輸血歴はなく、プリオン病の家族歴もなかった。2001年6月、48歳の時に漢字を書くことが困難になった。2001年10月、興奮性、人格変化、記憶障害など精神症状を発現、後に異常感覚、運動失調、認知症、異常行動が見られた。2002年8月のMRIでは、視床部にわずかに高信号が認められた。2003年1月、認知症、運動失調、過反射を示した。MRI画像は、視床部に対称性の高信号域を認めた。脳波画像(EEG)は徐波化を示したが、孤発性クロイツフェルト・ヤコブ病(sCJD)に特徴的な周期性同期性放電(PSD)は認められなかった。脳脊髄液の14-3-3蛋白質は陽性だった。コドン129(メチオニン/メチオニン)と219(グルタミン酸/グルタミン酸)に変異はなかった。この時点ではvCJD可能性例の診断基準を満たしていた。運動機能と認知機能は急速に低下した。

2003年12月、無動性無言、ミオクローヌス、錐体路徴候を示した。MRI像は、尾状核、被殻、視床、大脳皮質に高信号を認めた。信号強度は視床より尾状核と被殻の方が高かった。EEGはPSDを示しsCJDの可能性例と診断された。2004年12月、患者は肺炎で死亡した。剖検結果はvCJDの特徴を示した。前頭葉に花弁状空胞が見られ、プロテアーゼ抵抗性PrPは、Collingeの4型PrP^{Sc}、すなわちParchiの2B型だった。

これは日本初のvCJD確定例である。進行性の神経精神疾患はvCJDを示したが、罹病期間は通常(中央値14カ月)よりはるかに長かった。これまで、vCJDで視床枕徴候が陽性から陰性になった症例は複数報告があったが、PSDを伴うvCJD症例は初めてである。この症例は、PSDによってvCJDの可能性が除外されないことを示しており、生前診断でvCJDを見逃さないため、WHOのvCJDの定義の修正を提案する。今回の患者がどこで病原体に暴露したのかは明らかではない。患者の渡英時、英国でのBSE流行は続いており、このとき暴露の可能性がある食品を食べたことは確認されているが、フランスや他の国、または日本で感染した可能性も否定できない。英国で感染したと仮定すると、暴露から発症までの潜伏期間は11. 5年となる。

報告企業の意見	今後の対応
変異型クロイツフェルト・ヤコブ病(vCJD)日本初の症例は周期性脳波を示し、sCJDの可能性例と診断されたが、剖検結果はvCJDの特徴を示していたとの報告である。	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

解凍赤血球濃厚液「日赤」
照射解凍赤血球濃厚液「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

(12)

Case Report

The first Japanese case of variant Creutzfeldt-Jakob disease showing periodic electroencephalogram

Masahito Yamada on behalf of the Variant CJD Working Group, Creutzfeldt-Jakob Disease Surveillance Committee, Japan

Lancet 2006; 367: 874

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In February, 2004, a 50-year-old Japanese man was referred to our Creutzfeldt-Jakob disease (CJD) surveillance committee. In the first half of 1990, the patient had spent about 24 days in the UK, 3 days in France, and 2 weeks in other European countries where variant CJD (vCJD) has not been reported. He had no history of surgery or blood transfusion, or a family history of prion disease. In June, 2001, aged 48 years, he had difficulty in writing Chinese characters. In October, 2001, he showed mental symptoms, such as irritability, personality changes, and memory impairment, followed by painful dysaesthesia in the legs, ataxia, dementia, and abnormal behaviour. Retrospective review of an MRI taken in August, 2002, showed slight hyperintensity in the thalamus. In January, 2003, he showed dementia, ataxia, and hyperreflexia. Brain MRI at that time showed symmetrical hyperintensity of the thalamus. Electroencephalogram (EEG) showed diffuse slowing, but no periodic synchronous discharges (PSD). The cerebrospinal fluid was positive for 14-3-3 protein. Analysis of the prion protein (PrP) gene showed no mutation, methionine/methionine at codon 129, and glutamic acid/glutamic acid at codon 219. He showed rapid deterioration of both motor and cognitive function.

In December, 2003, he developed akinetic mutism, myoclonus, and pyramidal signs. Brain MRI showed hyperintensity in the caudate, putamen, thalamus, and cerebral cortex, with higher intensity in the caudate and putamen than the thalamus. EEG suggested the presence of PSD (figure, A). The diagnosis of probable sporadic CJD was supported by EEG and MRI findings.[1] He died of pneumonia in December, 2004. Autopsy showed findings characteristic of vCJD, including florid plaques (figure, B) and the Parchi type 2B or Collinge type 4 pattern of protease-resistant PrP (not shown).

This is the first Japanese case of definite vCJD. The progressive neuropsychiatric disorder was consistent with vCJD, although the illness duration was unusually long compared with most vCJD cases reported to date (median 14 months).[2] The findings 19 months after onset of symptoms, showing the pulvinar sign on MRI and the absence of PSD on EEG, together with the clinical features, fulfilled the criteria of probable vCJD.[3] However, 30 months after onset, PSD appeared on EEG, and the pulvinar sign on MRI disappeared following an increase in intensity of other grey matter nuclei, fulfilling the criteria of probable sCJD.[3] There have been no previous reports of PSD on EEG in vCJD, although conversion of a positive to negative pulvinar sign on MRI has been described in a few vCJD patients.[4] Our case shows that PSD does not exclude the possibility of vCJD. We suggest revision of the WHO vCJD case definition[3] to prevent missing cases of vCJD. It is unclear when our patient was exposed to the infective agent. The BSE outbreak in the UK was still increasing when he visited the UK, and it was confirmed that he ate food containing mechanically recovered meat that may be associated with contamination with BSE agent from nervous tissue;[5] however, exposure in France, other European countries, and Japan cannot be excluded. If he was exposed to the BSE agent in the UK (exposure just once would be sufficient to cause vCJD), we calculate the incubation period between such pinpoint exposure and onset of vCJD to be 11·5 years.

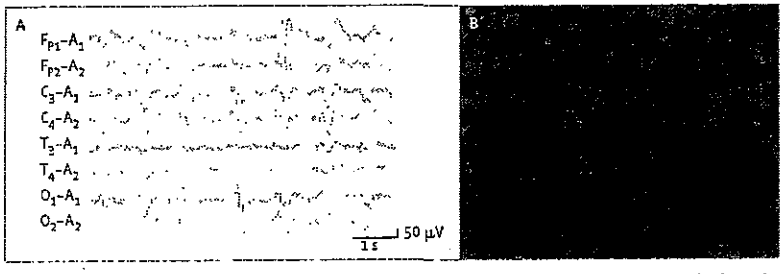

Figure: (A) EEG in August, 2004, 39 months after onset, showing PSD typical of sporadic CJD. (B) The frontal cortex showing florid plaques, severe spongiform changes, and neuronal loss (HE, bar=100 μm). PrP immunohistochemistry showed many PrP-positive plaques and PrP deposits with a pericellular pattern (not shown).

Contributors

Following the identification of this first Japanese case of vCJD by the CJD Surveillance Committee, Japan, we constituted the vCJD Working Group in the Committee. To protect the patient's privacy, we have decided to publish this report under the name of the vCJD Working Group, CJD Surveillance Committee, Japan. The corresponding author is the chair of both the vCJD Working Group and the CJD Surveillance Committee.

Acknowledgments

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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	①ポリエチレングリコール処理抗破傷風人免疫グロブリン ②乾燥抗破傷風人免疫グロブリン	研究報告の公表状況	The Lancet Neurology 2006; 5: 393-398	公表国 イギリス
販売名 （企業名）	①テタノブリンーIH（ベネシス） ②テタノブリン（ベネシス）			

研究報告の概要

＜背景＞
輸血を介したvCJD感染の可能性が確認されたことによって、ヒトの間でこの疾患が蔓延することへの懸念が起きている。我々が目指したのは、vCJD及びBSEの感染効率を比較し、人の感受性に関するコドン129遺伝的多型の影響の評価することを可能にするための医原的伝播のモデルを作成することであった。
＜方法＞
マウスPrP遺伝子の交換によってヒト又はウシのPrP蛋白を発現するマウスを作製した。ヒトのPrP遺伝子はコドン129にMM、VV、MVの遺伝型の多様性を有することから、コドン129のMM、VV、MV遺伝型を有するヒトPrPを発現するために、同じ遺伝背景を有する3つの近交系（inbred line）を作製した。マウスにBSE又はvCJDを接種し、疾患の臨床的及び病理学的な徴候を観察した。
＜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、各々の遺伝型で病理学的特徴が異なり、感染効率はMM＞MV＞VVの順であった。
＜解釈＞
BSEからヒトへの感染はおそらく、種の壁の存在によって制限を受けている。しかし、vCJDのヒトからヒトへの感染には、実質的に壁が低くなっているように見える。さらに、全ての個人は、コドン129の遺伝型に関係なく、輸血のようなルートによって、vCJDの2次感染が起こりやすい可能性がある。潜伏期間の長い疾患はこれらのモデルによって予測ができるが、このモデルは、病気の感染を更に拡げる危険性をあることを示しており、これにより重大な公衆衛生の問題を提示している。

使用上の注意記載状況・その他参考事項等

代表としてテタノブリンー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。

報告企業の意見	今後の対応
トランスジェニックマウスを用いたvCJD感染実験により、vCJDはコドン129がMM、VV、MVの全ての遺伝型が感染し、VV及びMVはMM型よりも潜伏期間が長いために、輸血のようなルートによって、vCJDの感染を更に拡げる危険性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。	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。

Predicting susceptibility and incubation time of human-to-human transmission of vCJD

M T Bishop, P Hart, L Aitchison, H N Baybutt, C Plinston, V Thomson, N L Tuzi, M W Head, J W Ironside, R G Will, J C Manson

Summary

Background Identification of possible transmission of variant Creutzfeldt-Jakob disease (vCJD) via blood transfusion has caused concern over spread of the disease within the human population. We aimed to model iatrogenic spread to enable a comparison of transmission efficiencies of vCJD and bovine spongiform encephalopathy (BSE) and an assessment of the effect of the codon-129 polymorphism on human susceptibility.

Methods Mice were produced to express human or bovine prion protein (PrP) by direct replacement of the mouse *PrP* gene. Since the human *PrP* gene has variation at codon 129, with MM, VV, and MV genotypes, three inbred lines with an identical genetic background were produced to express human PrP with the codon-129 MM, MV, and VV genotypes. Mice were inoculated with BSE or vCJD and assessed for clinical and pathological signs of disease.

Findings BSE was transmitted to the bovine line but did not transmit to the human lines. By contrast, vCJD was transmitted to all three human lines with different pathological characteristics for each genotype and a gradation of transmission efficiency from MM to MV to VV.

Interpretation Transmission of BSE to human beings is probably restricted by the presence of a significant species barrier. However, there seems to be a substantially reduced barrier for human-to-human transmission of vCJD. Moreover, all individuals, irrespective of codon-129 genotype, could be susceptible to secondary transmission of vCJD through routes such as blood transfusion. A lengthy preclinical disease is predicted by these models, which may represent a risk for further disease transmission and thus a significant public-health issue.

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Introduction

After the identification of variant Creutzfeldt-Jakob disease (vCJD) in 1996,[1] there have been many attempts to estimate the extent of the UK epidemic. Many individuals are likely to have been exposed to bovine spongiform encephalopathy (BSE) material through their diet; however, there have been only 161 cases of the disease in the UK. The predicted total number of future cases has ranged from the low hundreds[2] to hundreds of thousands.[3] However, findings from a retrospective immunocytochemical study that aimed to detect prion protein (PrP) in appendix and tonsil specimens suggested a prevalence of BSE infection of 237 per million people in the UK.[4] DNA sequence analysis of the PrP gene (*PRNP*) in vCJD has shown that 100% of tested cases are homozygous for methionine at the codon-129 polymorphism compared with about 40% of the general white population and about 70% of sporadic CJD cases. The methionine homozygous genotype (MM) has been included as a limiting variable in most mathematical predictions of the size of the epidemic.[2,4] Identification at autopsy of preclinical vCJD infection in a methionine/valine (MV) heterozygous individual who had received a transfusion of red cells from a donor who later died of vCJD, was the first indication that MM might not be the only susceptible genotype.[5]

Polymorphisms and mutations in *PRNP* in various species can affect disease susceptibility, although the precise mechanisms by which these effects are mediated have not been established.[6,7] Codon 129 of the human *PRNP* gene has been shown to affect the clinicopathological phenotype of disease in CJD and fatal familial insomnia.[6–13] Heterozygosity at *PRNP* codon 129, when compared with homozygous individuals, has been reported to lengthen incubation times in iatrogenic CJD cases associated with growth hormone treatment, and in kuru,[9,14] whereas valine homozygosity (VV) has been proposed to be protective for both BSE and vCJD transmission in studies that used murine models overexpressing human PrP.[15] At a molecular level, the biophysical properties of PrP refolding into the disease associated form (PrP^{Sc}) have been shown to be affected by the codon-129 genotype, with the methionine variant having an increased propensity to form PrP^{Sc}-like structures.[16]

We sought to analyse the transmission characteristics of BSE and vCJD to four inbred lines of transgenic mice after intracerebral inoculation with brain homogenate from cases of vCJD and BSE. We then aimed to use these models to address the apparent low level of vCJD in the human population resulting from exposure to BSE and to predict the potential for human-to-human spread of vCJD and the susceptibility of different genotypes in the human population.

Methods

Transgenic mice

Details of how the gene-targeted transgenic lines were created are supplied as supplementary information

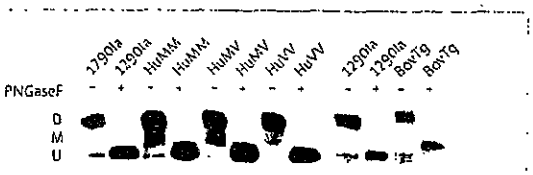

Figure 1: Western blot of brain extract from uninoculated mice showing that PrP^c is detected with equivalent electrophoretic mobility and glycoform ratio in all three human transgenic lines
D=diglycosylated PrP^c band; M=monoglycosylated PrP^c band; U=unglycosylated PrP^c band. In the BovTg line, a deglycosylated band is detected of increased molecular weight due to the additional N-terminal octapeptide repeat motif. Protein levels are similar to the wildtype line used in generating the transgenics (1290la). Glycosylation is confirmed by the reduction to a single band after deglycosylation with the enzyme PNGaseF. The anti-PrP antibody 7A12 was used for the HumTg blot as it will react with both murine and human PrP, and 8H4 was used for the BovTg blot.

See Online for webappendix

(webappendix). Transgenic mice were anaesthetised with halothane and then injected with 0·02 mL of brain homogenate into the right cerebral hemisphere. The vCJD tissue homogenate (at 10^{-2} dilution) was supplied by the UK National Institute for Biological Standards and Control (Code NHBY0/0003). BSE-infected cattle brain (Veterinary Laboratories Agency, reference BBP 12/92) was prepared by maceration of the tissue in sterile saline to a dilution of 10^{-1}. From 100 days they were scored each week for signs of disease.[17] Mice were killed by cervical dislocation whether they had clinical signs of transmissible spongiform encephalopathy (TSE) or another non-specific disorder. The brain was recovered at post mortem. Half the brain was snap-frozen in liquid nitrogen for biochemical analysis and the remaining half was fixed for histology.

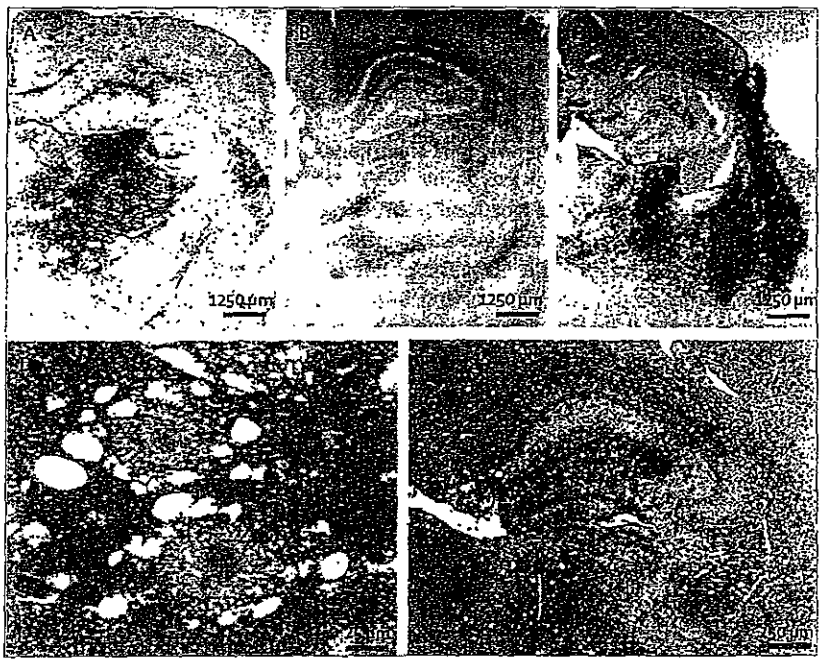

Figure 2: Immunocytochemistry of histological sections with anti-PrP antibody 6H4 showing the cortex, hippocampal, and thalamic regions of the mouse brain with PrP detection (brown)
A–D: Human transgenic mice with vCJD inoculum. A: HuMM mouse 693 days post inoculation. B: HuMV mouse 707 days post inoculation. C: HuVV mouse 693 days post inoculation. D: Florid plaques found in the hippocampus of the HuMM mouse in panel A. Each plaque has an eosinophilic core with a paler halo and is surrounded by a ring of vacuolation (haematoxylin and eosin stain). E: Hippocampal region of a BovTg mouse inoculated with BSE. PrP is deposited in a more diffuse/granular form with occasional plaques.

Procedures

Immunocytochemical detection of disease-associated PrP (PrP^{Sc}) deposits in the brain is a key pathological marker of TSE transmission, and variation in location and morphology of PrP^{Sc} deposits can be affected by both the strain of TSE agent and by the host PrP.[7,18] After fixation in 10% formal saline, brains were treated for 1·5 h in 98% formic acid (to reduce the titre of infectivity for safety reasons), cut transversely into four sections, and embedded in paraffin. We used the Vectastain Elite ABC Kit (Vector Labs, UK) with overnight primary antibody incubation (6H4 at 1:2000; Prionics, Switzerland) for PrP detection. Identification of antibody binding was through deposition of 3,3′-diaminobenzidine chromogen via a horseradish peroxidase reaction. The BSE-inoculated human transgenics were also studied using the Catalysed Signal Amplification kit (DAKO K1500). This kit uses the same principles as the Vector Labs kit, but has an additional step, which amplifies the final detected signal and therefore improves sensitivity.

Scoring of the abundance and location of TSE-associated vacuolation in grey and white matter of the brain is routinely used for diagnosis and strain classification in non-transgenic mice[17,19] and was used to assess all the mice in this study. TSE-related vacuolation was assessed at nine grey-matter regions and three white-matter regions to produce a lesion profile, as previously described.[20,21]

Analysis

Frozen brain samples from the human transgenic mice were homogenised in 0·9% saline to give a 10% suspension. This material was cleared by centrifugation and the supernatant treated with 0·05 g/L proteinase K for 1 h at 37°C, as previously described in detail.[22] The digested product was denatured then loaded onto a 10% Bis/Tris NuPAGE Novex gel (Invitrogen, UK). After electrophoresis the gel was blotted onto polyvinylidine difluoride (PVDF) membrane. We used the ECL+ technique (Amersham Biosciences, UK) with primary antibody 6H4 (Prionics, Switzerland) at 1:40000 and an anti-mouse IgG peroxidase-linked secondary (Amersham Biosciences, UK) at 1:40000 for the detection of PrP. Chemiluminescence was captured on radiographic film. Samples prepared for figure 1 were digested overnight at 37°C with 500 units of PNGaseF (New England Biolabs, UK) and not with proteinase K; the primary antibody was 7A12.[23]

Frozen brain samples from the bovine transgenic mice were homogenised in an NP40 buffer (0·5% v/v NP40,

0·5% w/v sodium deoxycholate, 0·9% w/v sodium chloride, 50mM Tris-HCl pH 7·5) to give a 10% suspension. This material was cleared by centrifugation and the supernatant digested with PNGaseF. The products were denatured then loaded onto a 12% Novex Tris/Glycine gel (Invitrogen, UK). After electrophoresis the gel was blotted onto PVDF membrane. PrP was identified with the SuperSignal West Dura chemiluminescence detection kit (Pierce, UK) with primary antibody 8H4[14] at 1:20000 and an anti-mouse IgG peroxidase-linked secondary (Jackson Immuno Research Laboratories, UK) at 1:10000. Images were captured on radiographic film and with a Kodak 440CF digital imager (figure 1).

Role of the funding source

The sponsors of this 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

We first investigated the potential effects of the species barrier between BSE and human beings and any alteration in that barrier once BSE had passed through people in the form of vCJD. We then investigated the effect of the codon-129 polymorphism on human-to-human transmission of vCJD using gene-targeted inbred mice developed by direct replacement of the murine *PrP* gene for the human gene. These mice produce PrP under the control of the normal regulatory elements for PrP and thus express physiological concentrations of PrP with the correct tissue distribution (figure 1). Three inbred lines with an identical genetic background were produced to express human PrP with the codon-129 MM, MV, and VV genotypes (designated HuMM, HuMV, and HuVV, respectively). Each line differs by only a single codon in *PRNP* and in all other respects the mice were genetically identical. Additionally, in an identical manner, we produced mice that express bovine PrP to enable direct comparisons to be made not only between transgenic and wild-type mice, but also between each of the transgenic lines.

Typical clinical signs of TSE disease were seen in more than half (15/22) the BovTg mice inoculated with BSE material with a mean incubation period of 551 days (SD 47). These clinical cases were confirmed by a positive test for the presence of TSE vacuolation or PrP^{Sc} deposition by immunocytochemistry. The lesion profiles generated for targeting and degree of vacuolation showed similar patterns for all positive mice. Immunocytochemical data showed PrP^{Sc} deposition mainly in a diffuse and synaptic form, and also as plaque-like structures, frequently associated with areas of spongiform change (figure 2). Deposition was most abundant in the thalamus and hippocampus, but was recorded throughout other regions of the brain. The cerebral cortex showed only occasional plaque-like structures and the cerebellum had only a few areas of PrP^{Sc} deposition limited to the granule cell layer. Further pathological analysis was undertaken on mice that were culled for reasons other than clinical TSE (intercurrent deaths). This analysis showed that all the brains had pathological signs of TSE disease in terms of vacuolation or PrP deposition. Thus, all the bovine transgenic mice (22/22) seemed to be susceptible to BSE infection, although not all developed clinical signs of infection (tables 1 and 2).

HuMM, HuMV, and HuVV mice were inoculated with BSE material and after extensive pathological analysis all were confirmed as negative for TSE transmission (table 1). Mice of each genotype line were inoculated with vCJD material. Two pathologically confirmed clinically positive mice were seen in the HuMM line (at 497 and 630 days post inoculation), one in the HuMV line (at

	Clinically positive	Vacuolation positive	PrP positive*	Negative†
BovTg (n=22)				
0–400	0	3	6	0
401–500	1	1	0	0
501–600	10	11	5	0
>600	4	4	2	0
HuMM (n=18)				
0–400	0	0	0	4
401–500	0	0	0	5
501–600	0	0	0	2
>600	0	0	0	7
HuMV (n=23)				
0–400	0	0	0	3
401–500	0	0	0	6
501–600	0	0	0	4
>600	0	0	0	10
HuVV (n=22)				
0–400	0	0	0	9
401–500	0	0	0	4
501–600	0	0	0	7
>600	0	0	0	2

*Because most mice were positive by both clinical and vacuolation scoring not all mice were tested by immunocytochemistry for PrP deposition. †Negative by clinical or pathological analysis, or positive by clinical scoring but not confirmed by pathology.

Table 1: **Clinical and pathological scoring of BovTg and human transgenic mice, by number of days after BSE inoculation**

	BSE				vCJD		
	BovTg	HuMM	HuMV	HuVV	HuMM	HuMV	HuVV
Susceptibility*	22/22	0/18	0/23	0/22	11/17	11/16	1/16

*Positives confirmed by immunocytochemistry or lesion profile.

Table 2: **Susceptibility to TSE disease comparison of BovTg and human transgenic mice inoculated with BSE or vCJD**

	Clinically positive	Vacuolation positive	PrP positive	Negative*
HuMM (n=17)				
0-400	0	0	2	2
401-500	1	1	1	2
501-600	0	1	3	2
>600	1	4	5	0
HuMV (n=16)				
0-400	0	0	0	0
401-500	0	0	0	0
501-600	0	0	4	3
>600	1	1	7	2
HuVV (n=16)				
0-400	0	0	0	0
401-500	0	0	0	1
501-600	0	0	0	5
>600	0	1	1	9

*Negative by clinical or pathological analysis, or positive by clinical scoring but not confirmed by pathology.

Table 3: Clinical and pathological scoring of human transgenic mice, by number of days after vCJD inoculation

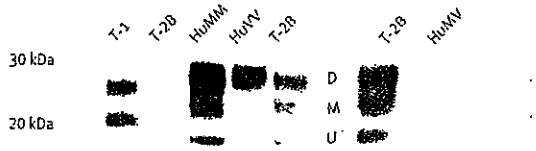

Figure 3: Western blots of brain extract from three transgenic lines inoculated with vCJD
D=diglycosylated PrP^{Sc} band; M=monoglycosylated PrP^{Sc} band; U=unglycosylated PrP^{Sc} band. T-2B corresponds to human vCJD brain homogenate showing the typical PrP^{Sc} type 2B and T-1 corresponds to human sCJD brain homogenate showing the typical PrP^{Sc} type 1 signature. Type 2B and 1 differ in mobility of the unglycosylated band (~19 kDa and ~20 kDa respectively) and the degree of glycosylation (diglycosylated dominant and mono/unglycosylated dominant respectively). All samples were treated with proteinase K. The anti-PrP detection antibody was 6H4. The HuMV and T2-B control blot had to be overexposed as the signal from the HuMV was weak, due to the low levels of PrP^{Sc} seen by immunocytochemistry.

665 days post inoculation), and none in the HuVV line (table 3). HuMM mice were more likely to show disease-associated vacuolation, beginning at around 500 days post inoculation. Six were scored positive and showed similar distribution of vacuolation in the brain, with the highest levels found in the dorsal medulla, thalamus, and cerebellar white matter. By contrast, only a single mouse in each of the HuMV and HuVV groups scored positive for vacuolation at approximately 700 days post inoculation.

Most of the HuMM mice (11/15) showed PrP^{Sc} deposition in most areas of the brain at a relatively early stage (from around 370 days post inoculation), before the vacuolar pathology became evident. From 500 days post inoculation the appearance of vacuolation was accompanied by a significant increase in PrP^{Sc} deposition. By contrast, although PrP^{Sc} deposition was identified in many HuMV mice (11/13), they had little deposition restricted to only a few areas (including the ventrolateral and ventromedial thalamic nuclei and the red nucleus of the mid-brain), even after 700 days post inoculation (figure 2, table 4). Although PrP^{Sc} deposition was clearly present at 581 days, the timing of initial onset of deposition in this line was not established.

Significant levels of PrP^{Sc} deposition were noted in the brain of the subclinical HuVV case. Indeed, these were similar in intensity to those observed in the clinical HuMM cases. Patterns of PrP deposition and plaque formation show differences among the three genotypes, including the presence of florid plaques only in the HuMM mice (table 4).

PrP^{Sc} found in vCJD brain is characterised by a 19 kDa non-glycosylated fragment and the predominance of the diglycosylated form (type 2B).[22] Both biochemical properties of PrP^{Sc} are maintained when vCJD is transmitted to the human transgenic mice, irrespective of their codon-129 genotype (figure 3). Preliminary densitometric analysis suggested that there was an increase in the diglycosylated form in the HuVV mouse compared with the HuMM mouse. Additionally, comparison of PrP^{Sc} from the BSE inoculum and brain material from BovTg mice also confirmed propagation of the predominantly diglycosylated glycoform signature of PrP^{Sc} associated with the BSE/vCJD agent strain (data not shown).

	HuMM	HuMV	HuVV
Vacuolation*	Thalamus (severe); cerebral cortex and hippocampus (mild); cerebellar cortex (minimal)	Thalamus, cerebral cortex, hippocampus, and cerebellar cortex (minimal)	Thalamus and cerebral cortex (severe); hippocampus (mild); cerebellar cortex (minimal)
Plaque formation*	Fibrillary amyloid plaques; florid and non-florid plaques in cerebral cortex and hippocampus; no evidence of plaques in cerebellum	No evidence of amyloid plaques	Amorphous non-fibrillary structures often forming into clusters in cerebral cortex and thalamus
PrP deposition†	Intense staining of plaques in hippocampus and cerebral cortex; plaque-like, pericellular, and amorphous deposits in the hippocampus; synaptic, peri-neuronal, and diffuse perivascular deposits in the thalamus	Occasional small plaque-like deposits and pericellular deposits in the thalamus	Strongly positive large amorphous deposits and clusters of plaques, small plaque-like structures, perivascular aggregates, and sub-pial deposits in the cerebral cortex and thalamus

*Analysed with haematoxilin and eosin staining. †Analysed with immunocytochemical techniques.

Table 4: Comparison of TSE-associated neuropathology in human transgenic mice inoculated with vCJD

Discussion

Although the cattle BSE epidemic in the UK has amounted to more than 180 000 cases since the 1980s, the extent of the human vCJD epidemic has so far remained limited with the total number of cases worldwide currently at 190. One explanation for this apparent discrepancy is that there exists a significant species barrier between cattle and human beings, which limits the susceptibility of the human population to BSE. The data shown here suggest that this could indeed be the case since BSE was readily transmissible to the bovine transgenic mice but not to the human transgenic mice. However, once BSE has passed through human beings in the form of vCJD, the transmissibility of this TSE strain is altered for the human population.

All the human transgenic lines inoculated with BSE were negative for TSE transmission, which suggests that either the human transgenic lines are relatively resistant to transmission of BSE or the incubation time is longer than the length of the experiment (approximately 700 days). BSE transmission previously observed by others, in human transgenic lines overexpressing the human prion protein, could be due to overexpression of the *PrP* gene and may not therefore give a true reflection of the species barrier between BSE and human beings.[15,25,26] This apparent resistance of human transgenic mice to BSE could be explained by a large species barrier and this in turn could explain the low number of vCJD cases in the human population.

vCJD was transmitted to all three human lines with different pathological characteristics for each genotype, and a gradation of transmission efficiency from MM to MV to VV. The greater transmission efficiency in HuMM mice suggests that homozygosity for methionine at codon 129 leads to earlier onset of TSE-related pathological features and clinical disease than for the other two genotypes. The differences in PrP^{Sc} deposition in the HuMM and HuMV lines suggest that the codon-129 polymorphism in human beings is likely to affect the distribution of PrP^{Sc} deposition in the brain. Moreover, the similar numbers that scored positive for PrP deposition in each of the MM and MV groups (11/15 and 11/13 respectively) suggest that the two genotypes might be equally susceptible to vCJD, but with different incubation periods. Titration experiments are needed to fully compare the susceptibility of each line. The single HuVV mouse positive for PrP^{Sc} shows that VV individuals may be susceptible to vCJD with very long incubation times, including a lengthy subclinical phase. Transmission studies from all three genotype mice are now underway to examine the infectious nature of the disease and determine any alterations in the strain characteristics on passage through human transgenic mice. By contrast with published data suggesting that VV individuals cannot propagate the vCJD biochemical phenotype,[15] the data presented here suggest that the PrP^{Sc} type will remain a useful diagnostic feature of secondary vCJD infection irrespective of codon-129 genotype, as has been observed for the two extant cases of transfusion-associated vCJD infection.[1,2]

Transmission of vCJD to the three lines of human transgenic mice indicates that the human population could be at significantly heightened risk of developing disease after iatrogenic exposure to vCJD. Secondary transmission of vCJD has partly removed the cattle-to-human species barrier and has resulted in an agent that can be transmitted from human to human with relative efficiency. Transmission studies in cynomolgus macaques provide further evidence for this agent adaptation as they show reduction in incubation times after serial passage of BSE.[26] Our BSE inoculation at 10^{-1} dilution was compared with vCJD inoculation at 10^{-2} because the latter inoculum was found to be toxic to the mice at 10^{-1}. Use of a higher dose of vCJD inoculum would have maintained or increased the transmission efficiency of vCJD and enhanced the current findings.

Our findings raise concerns relevant to the possibility of secondary transmission of vCJD through blood transfusion, fractionated blood products, or contaminated surgical instruments. For this study mice were injected intracerebrally, whereas the probable human exposure to these agents is by peripheral routes (eg, oral or intravenous), and thus human-to-human exposures might be significantly less efficient. However, it is difficult to know for sure what the practical implications might be in human beings. Peripheral route challenge is in progress; however, BSE transmission studies in primates have shown the intravenous route to be as efficient as the intracerebral route, with an extension of the incubation time.[26]

Although all cases of vCJD up to now have been observed in the MM genotype, this model of human-to-human vCJD transmission suggests that other genotypes are also susceptible. In our experimental setting, all *PRNP* codon-129 genotypes are susceptible to vCJD infection; however, progressive development of pathological TSE features (vacuolation and PrP deposition) is more rapid in the MM-genotype mice. An explanation for this finding might be provided by in-vitro conversion of recombinant human PrP by BSE and vCJD agents, which has shown that PrP with methionine at position 129 is more efficiently converted than PrP with valine, and that conversion by vCJD is significantly more efficient than by BSE.[29] Long incubation periods during which PrP^{Sc} is deposited predicts that, in human beings, infection could be present in all genotypes for a significant period before clinical onset. Incubation periods of more than 30 years have been reported in the human TSE disease kuru.[30]

The possibility that an MV or VV genotype could result in a phenotype distinct from that recognised in vCJD draws attention to the importance of systematic assessment of the clinical, genetic, pathological, and

biochemical features of all human prion diseases. Our findings indicate that for human-to-human vCJD infection it should be assumed that all codon-129 genotype individuals (not just MM) can be infected, that long incubation times can occur, and that a significant level of subclinical disease might be present in the population.

Contributors

MTB, PH, and CP did immunocytochemical and western blot analysis; JCM, NT, HNB, and LA produced the transgenic mouse lines; JWI supplied vCJD case material and reviewed the neuropathology; VT did the mouse inoculations; and MTB, PH, MWH, RGW, JWI, and JCM prepared the manuscript.

Conflicts of interest

We have no conflicts of interest.

Acknowledgments

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研究報告の概要

確認されていない環境中の感染性の貯蔵場所が、ヒツジ、シカ及びエルクのプリオン病（TSE）の自然感染の一因となっている。プリオンの感染性は、病気の動物の畜殺及び感染した死骸の腐敗を通して土壌環境に入る可能性がある。廃棄手段として、TSE 汚染のウシ、ヒツジ及びシカを埋めることにより、地表下への意図しない混入が起こる。我々は、ありふれた土壌ミネラルと病気に関係するプリオン蛋白の相互作用を検討することによって、土壌がTSE貯蔵場所として作用する可能性を調査した。
本調査で、2種類の粘土ミネラル（モンモリロナイト及びカオリナイト）、石英及び4種類の無処理土壌サンプルに PrP^{Sc} が吸着することがわかった。加えて、モンモリロナイトと PrP^{Sc} の吸着は強固であり、低pH（pH2.5）並びに高pH（pH11.5）、イオン強度の増加（0.1M又は1.0M NaCl）及び水素結合を切断し土壌鉱物からたんぱく質の分離させるのに有効であるカオトロピック剤（8M尿素及び8Mグアニジン）でも検出可能な PrP^{Sc} を遊離することができず、10%SDS存在下の100℃煮沸のみ PrP^{Sc} を遊離することができた。モンモリロナイトから分離した PrP^{Sc} はN末端で切れていた。また、モンモリロナイトに吸着した PrP^{Sc} は感染実験により感染性を維持していることが確認できた。我々の研究結果は、土壌環境に放出された PrP^{Sc} 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。

使用上の注意記載状況・その他参考事項等

代表としてテタノブリンー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。

報告企業の意見	今後の対応
土壌環境に放出されたPrP^{Sc}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ことにより、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。	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。

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Prions Adhere to Soil Minerals and Remain Infectious

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***An unidentified environmental reservoir of infectivity contributes to the natural transmission of prion diseases* (transmissible spongiform encephalopathies [TSEs]) in sheep, deer, and elk. Prion infectivity may enter soil environments via shedding from diseased animals and decomposition of infected carcasses. Burial of TSE-infected cattle, sheep, and deer as a means of disposal has resulted in unintentional introduction of prions into subsurface environments. We examined the potential for soil to serve as a TSE reservoir by studying the interaction of the disease-associated prion protein (PrP^{Sc}) with common soil minerals. In this study, we demonstrated substantial PrP^{Sc} adsorption to two clay minerals, quartz, and four whole soil samples. We quantified the PrP^{Sc}-binding capacities of each mineral. Furthermore, we observed that PrP^{Sc} desorbed from montmorillonite clay was cleaved at an N-terminal site and the interaction between PrP^{Sc} and Mte was strong, making desorption of the protein difficult. Despite cleavage and avid binding, PrP^{Sc} bound to Mte remained infectious. Results from our study suggest that PrP^{Sc} released into soil environments may be preserved in a bioavailable form, perpetuating prion disease epizootics and exposing other species to the infectious agent.**

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Introduction

Transmissible spongiform encephalopathies (TSEs, prion diseases) are a group of fatal neurodegenerative diseases that affect a variety of mammalian species and include bovine spongiform encephalopathy (BSE, "mad cow" disease), chronic wasting disease (CWD) of deer and elk, sheep scrapie, and Creutzfeldt-Jakob disease in humans [1]. The agricultural, economic, and social impacts of prion diseases have been intensified by evidence suggesting transmissibility of BSE to humans [2]. The putative infectious agent in these diseases, designated PrP^{Sc}, is a misfolded isoform of the normal cellular prion protein (PrP^{C}). The amino acid sequences of PrP^{Sc} and PrP^{C} are identical [3]; normal and abnormal forms of the protein differ only in conformation. No differences in posttranslational covalent modification have been demonstrated [3]. Circular dichroism and infrared spectroscopy indicate that the disease-specific isoform has a higher β-sheet and lower α-helix content than PrP^{C} [4]. The normal isoform is soluble and primarily monomeric in solution, whereas PrP^{Sc} forms insoluble aggregates.

Sheep scrapie and cervid CWD are unique among TSEs, because epizootics can be sustained by horizontal (animal-to-animal) transmission [5,6]. Routes of natural transmission remain to be clarified, but available evidence indicates that an environmental reservoir of infectivity contributes to the maintenance of these diseases in affected populations [6–8]. The expanding range of CWD (several regions of North America and Korea) increasingly brings domestic livestock, companion animals, and wildlife species into contact with infected animals and carcasses, and shedded TSE agent, raising the possibility of cross-species transmission. This was demonstrated by the recent detection in Colorado, USA, of a free-ranging, CWD-infected moose, a species not previously known to be affected by the disease in the wild [9].

Although other modes of environmental transmission of scrapie and CWD have been proposed (e.g., flesh flies [10], hay mites [11]), several lines of evidence point to soil as a reservoir for TSE infectivity. TSE infectivity exhibits remarkable resistance to inactivation by most chemical agents, radiation, and heat [12] and has been shown to persist after burial in soil for at least 3 y [13]. Anecdotal observations of healthy sheep contracting scrapie after occupying fields previously containing diseased animals have been reported [7,8]. Although these older studies did not account for the genetic susceptibility of the sheep under study, they suggest that scrapie agent can persist in the environment for years. Recent controlled field experiments provide more compelling evidence of the environmental persistence of prions. Miller et al. [14] demonstrated that naïve mule deer could contract CWD

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Abbreviations: BH, brain homogenate; BSE, bovine spongiform encephalopathy; CWD, chronic wasting disease; dpi, days postinoculation; Kte, kaolinite; Mte, montmorillonite; PK, proteinase K; PrP^{C}, normal cellular isoform of the prion protein; PrP^{Sc}, disease-associated prion protein; TSE, transmissible spongiform encephalopathy

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Synopsis

Transmissible spongiform encephalopathies (TSEs) are a group of incurable diseases likely caused by a misfolded form of the prion protein (PrP^{Sc}). TSEs include scrapie in sheep, bovine spongiform encephalopathy ("mad cow" disease) in cattle, chronic wasting disease (CWD) in deer and elk, and Creutzfeldt-Jakob disease in humans. Scrapie and CWD are unique among TSEs because they can be transmitted between animals and the disease agents appear to persist in environments previously inhabited by infected animals. Soil has been hypothesized to act as a reservoir of infectivity, because PrP^{Sc} likely enters soil environments through urinary or alimentary shedding and decomposition of infected animals. In this manuscript, the authors test the potential for soil to serve as a reservoir for PrP^{Sc} and TSE infectivity. They demonstrate that PrP^{Sc} binds to a variety of soil minerals and to whole soils. They also quantitate the levels of protein binding to three common soil minerals and show that the interaction of PrP^{Sc} with montmorillonite, a common clay mineral, is remarkably strong. PrP^{Sc} bound to Mte remained infectious to laboratory animals, suggesting that soil can serve as a reservoir of TSE infectivity.

when housed in paddocks previously inhabited by infected animals or containing decomposed infected carcasses.

TSE agents directly enter the environment when carcasses of infected animals decompose [13], through alimentary shedding of the agent from gut-associated lymphoid tissue [15,16], or from urinary excretion from infected, nephritic animals [17]. Furthermore, bovine, sheep, and deer TSE agents have been introduced to soil environments through the burial of diseased carcasses and other infected material [18]. Animals ingest soil both deliberately and incidentally [19]. Cattle, deer, sheep, and other animals can consume hundreds of grams of soil daily [20,21]. Taken together, these data support the notion that PrP^{Sc}-contaminated soil may allow intraspecies TSE transmission and enhance the likelihood of spread to other species. As a first step toward understanding the role of soil as a reservoir of TSE infectivity, we investigated the binding of PrP^{Sc} to common soil minerals and whole soils and examined the infectivity of mineral-bound prions.

Results

Binding of PrP^{Sc} to Soil Minerals

We examined the sorption of purified PrP^{Sc} to three common soil minerals (Table S1): quartz, montmorillonite (Mte, an expandable layered silicate clay mineral), and kaolinite (Kte, a nonexpandable phyllosilicate mineral). Quartz of two particle sizes was employed in sorption experiments: fine sand (hydrodynamic diameter [d_h] = 125–250 μm), representing quartz concentrated in the sand and silt fractions of soils, and microparticles (d_h = 1–5 μm), representing quartz present in the coarse clay fraction [22]. Purified PrP^{Sc} (~0.2 μg) was introduced into aqueous suspensions (pH 7.0) of each soil mineral and subjected to 2-h mixing. Unbound PrP^{Sc} was separated from bound protein by centrifugation through a 750-mM sucrose cushion. Bound and unbound fractions were analyzed by SDS-PAGE and immunoblotting.

The extent of PrP^{Sc} sorption differed among the mineral particles examined. All detectable PrP^{Sc} adsorbed to the expandable clay mineral Mte (Figure 1A). X-ray diffraction analysis provided no evidence that PrP^{Sc} entered Mte interlayer spaces (Mte d_{001} spacings were 1.22 nm and 1.47 nm before and after PrP^{Sc} adsorption, respectively); prion protein appeared to adsorb to only external clay surfaces. PrP^{Sc} did not associate with an equal mass of fine quartz sand at levels detectable by immunoblotting (Figure 1A). A large degree of PrP^{Sc} binding to the nonexpandable clay mineral Kte was observed when the surface area was matched to that of external Mte surfaces (Figure 1A). The limited association of PrP^{Sc} with fine quartz sand was at least in part attributable to the much smaller specific surface area of these particles as compared to kaolinite and external Mte surfaces (Table S1). When quartz surface area was matched to that of external Mte surfaces, all detectable PrP^{Sc} adsorbed to quartz (Figure 1A).

Adsorption Capacities of Soil Minerals for PrP^{Sc}

The amount of PrP^{Sc} adsorbed to Mte was semiquantitatively assessed by serial dilution of samples to the limit of immunoblotting detection. The dilution at which no detectable immunoreactivity remained provided a basis for comparison with samples lacking immunoreactivity before dilution. PrP^{Sc} desorbed from Mte still exhibited immunoreactivity after 100-fold dilution, indicating that the amount of prion protein adsorbed to Mte exceeded that in samples without immunoreactivity (e.g., unbound PrP^{Sc} in experiments with Mte) by at least two orders of magnitude (Figure 1B). Furthermore, this result suggests that fine quartz sand was saturated by at least 100-fold less PrP^{Sc} (≤ 0.002 μg) than used for sorption experiments (Figure 1A).

To assess the PrP^{Sc}-binding capacity of the other soil minerals, increasing quantities of PrP^{Sc} were added to each mineral. Protein desorbed from mineral particles was serially

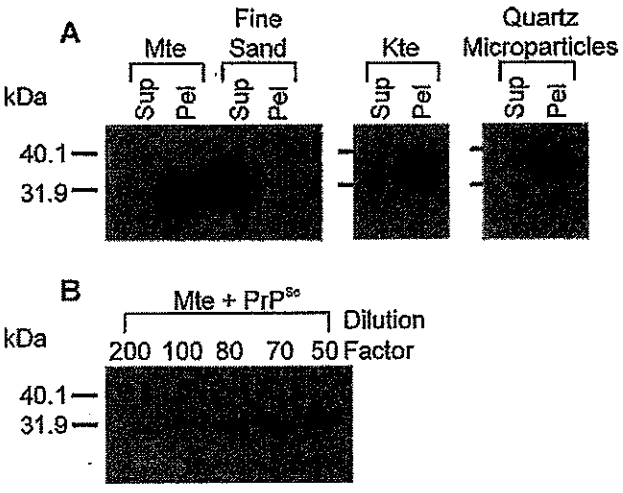

Figure 1. PrP^{Sc} Adsorption to Clay Minerals and Quartz Microparticles Substantially Exceeded That to Fine Quartz Sand

(A) Detectable amounts of PrP^{Sc} adsorbed to Mte and Kte but not to fine quartz sand (d_h = 125–250 μm). PrP^{Sc} desorbed from Mte was of lower molecular mass than the starting material. Adsorption to quartz was observed when quartz microparticles (d_h = 1–5 μm) were employed and surface area was matched to Mte.

(B) Immunoblotting sensitivity was determined by dilution of Mte-adsorbed PrP^{Sc} to the limit of detection. Protein was desorbed from Mte in 50 μl of SDS-PAGE sample buffer at 100 °C and serially diluted. Immunoblots used monoclonal antibody (mAb) 3F4. Pel, PrP^{Sc} associated with pelleted mineral particles; Sup, unbound PrP^{Sc} in supernatant.

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diluted and subjected to SDS-PAGE and immunoblotting to semiquantitate the amount of sorbed protein. The binding capacity of a mineral was attained when subsequent PrP^{Sc} additions did not further increase the dilution factor required to reach the limit of immunoblotting detection (Table 1). Of the minerals examined, Mte exhibited the highest PrP^{Sc} adsorption capacity (~100 $\mu g_{protein}\ mg_{Mte}^{-1}$). The adsorption capacity of the quartz microparticles was nearly 10-fold less (~15.6 $\mu g_{protein}\ mg_{microparticle}^{-1}$), and that of Kte was nearly 100-fold less than Mte (~2 $\mu g_{protein}\ mg_{Kte}^{-1}$). When expressed on a surface-area basis (Table 1), the adsorption capacities of Mte and quartz microparticles were indistinguishable by our measurement method; that of Kte was 25 times less. These data demonstrate that mineral surface properties contribute to differences in the amount of PrP^{Sc} bound.

PrP^{Sc} Desorbed from Mte Surfaces Is Cleaved

Unexpectedly, PrP^{Sc} desorbed from Mte surfaces exhibited a lower molecular mass (~27–31 kDa) than the starting material (~33–35 kDa) (Figure 1A). Neither contaminant proteases nor metal oxide coatings on Mte particles appeared responsible for PrP^{Sc} cleavage, as treatments to counteract each did not prevent cleavage (unpublished data). Prior to sorption experiments, Mte was boiled in a solution of 10 mM NaCl for 10 min to denature contaminant proteases, or binding experiments were performed in the presence of a cocktail of protease inhibitors to inactivate them. Neither treatment prevented PrP^{Sc} cleavage. Amorphous metal oxide coatings on clay mineral particles can alter their surface reactivities and could potentially be responsible for PrP^{Sc} cleavage. The size-fractionated Mte used in this study has been reported to not contain such impurities at levels detectable by X-ray diffraction analysis [23], and precautionary pretreatment of the clay with a buffered neutral citrate-bicarbonate-dithionate solution to remove metal oxide coatings [24] failed to prevent cleavage.

Prion protein desorbed from Kte and quartz did not exhibit a change in molecular mass (Figure 1A), suggesting that surface properties specific to Mte were responsible for the cleavage. Previous studies on protein interaction with Mte have not noted reductions in molecular mass upon desorption [25,26]. We incubated PrP^{Sc} with Mte for short time periods (1–15 min) to qualitatively investigate initial adsorption and cleavage kinetics. Adsorption of PrP^{Sc} to Mte was apparent within 1 min, and reduction in protein molecular mass was discernable (Figure 2A). Prion protein cleavage consistently occurred early within the first 15 min of contact with Mte and appeared maximal by 60 min. Cleavage of PrP^{Sc} caused by sorption to or desorption from Mte seemed to be a phenomenon specific to this protein. We examined sorption and desorption of scrapie-infected hamster brain homogenate (BH) to Mte. Desorption of brain proteins from Mte produced no changes in the overall molecular mass distribution as visualized by Coomassie blue staining (unpublished data). Subunit C2 of the 20S proteasome (~29 kDa), an unrelated protein similar in size to PrP likewise did not appear cleaved upon desorption from Mte (Figure 2B). In contrast, PrP^{Sc} in BH was cleaved (Figure 2C).

Cleavage of PrP^{Sc} involved loss of the N-terminal portion of the protein, which is not necessary for infectivity [3]. Prion protein desorbed from Mte lost immunoreactivity with an antibody directed against amino acids 23–37 on the protein N terminus, indicating that all or part of the epitope of this antibody was missing from the desorbed protein (Figure 2D).

Table 1. PrP^{Sc} Adsorption Capacities for the Minerals Examined[a]

Mineral	Binding Capacity (Sorbent Mass Basis) ($\mu g_{protein}\ mg_{mineral}^{-1}$)	Binding Capacity (Sorbent Surface Area Basis) ($mg_{protein}\ m_{mineral}^{-2}$)
Mte	87–174	2.8–5.7
Kte	1.7–2.6	0.15–0.22
Quartz microparticles	13.6–27.1	2.7–5.4

[a]Protein concentration determined by Bradford assay; PrP^{Sc} concentration was taken as 87% of total protein [45]. Reported adsorption capacities represent upper estimates, as the fraction of PrP^{Sc} in clarified preparations may have been lower.

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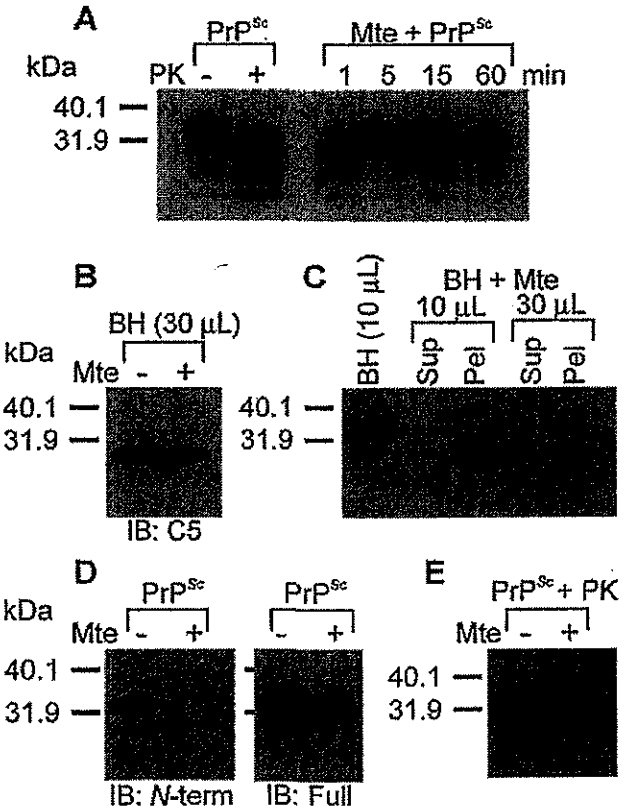

Figure 2. PrP^{Sc} Desorbed from Mte Is Cleaved

(A) PrP^{Sc} cleavage occurs after short contact times with Mte surfaces.
(B) The molecular mass protein C2 of the 20S proteasome subunit from BH was unaltered following desorption from Mte.
(C) Cleavage of PrP^{Sc} present in infected BH was apparent after desorption from Mte.
(D) PrP^{Sc} desorbed from Mte lost immunoreactivity against an antibody recognizing the N-terminal portion of the mature protein.
(E) PrP^{Sc} pretreated with PK bound to Mte and did not exhibit further reduction in molecular mass when desorbed.
Immunoblots (A, B, and E) used mAb 3F4. Immunoblots (C and D) employed anti-C2 and R20 polyclonal antibodies, respectively. Pel, PrP^{Sc} associated with pelleted mineral particles; Sup, unbound PrP^{Sc} in supernatant.

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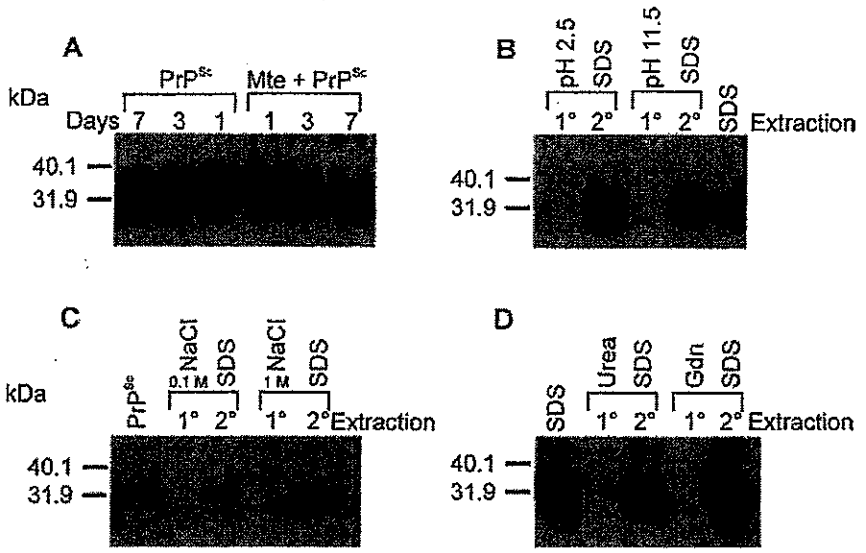

Figure 3. PrP^{Sc} Adsorbed to Mte Avidly and Remained Stable

(A) PrP^{Sc} was stable when adsorbed to Mte for at least 7 d. (B) Extremes in pH (100 mM phosphate at pH 2.5 or 11.5), (C) sodium chloride (100 mM or 1 M), and (D) chaotropic agents (8 M urea or 8 M guanidine [Gdn]) did not desorb detectable amounts of PrP^{Sc} from Mte. Primary extractions (1°) were followed by secondary extractions (2°) extractions with a 10% SDS solution at 100°C. Immunoblots (A–D) employed mAb 3F4. Pel, PrP^{Sc} associated with pelleted mineral particles; Sup, unbound PrP^{Sc} in supernatant.

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In contrast, probing identical samples with a polyclonal antibody against full-length PrP demonstrated that PrP^{Sc} was desorbed from the Mte. Although the precise cleavage site was not determined, these data suggest that the N terminus of PrP^{Sc} was removed; the fate of the cleaved amino acid residues is not known, as they may have remained bound to the clay or may have been extracted but not detected. When the N-terminal ~70 amino acids were removed from PrP^{Sc} by pretreatment with proteinase K (PK) prior to adsorption to Mte, we observed sorption to the Mte, but no further reduction in molecular mass upon desorption, evidence that other regions of the protein remain intact when associated with Mte (Figure 2E). These results also indicate that the N terminus of PrP^{Sc} is not necessary for adsorption to Mte.

Strength of PrP^{Sc} Binding to Mte

PrP^{Sc} attachment to Mte was avid, and sorbed PrP^{Sc} was stable. Washing Mte-PrP^{Sc} with the background solution used in sorption experiments did not induce detachment of detectable amounts of PrP^{Sc} from Mte (unpublished data). Contact of PrP^{Sc} with Mte for up to 1 wk did not result in additional degradation, indicating that the protein was not rendered more susceptible to cleavage by further structural rearrangements on the clay surface (Figure 3A). The strength of PrP^{Sc} attachment to Mte was surprising, even in light of reports of protein sorption-desorption hysteresis on mineral surfaces [26]. Conditions previously employed to desorb other proteins from soil minerals were largely ineffective in detaching PrP^{Sc} from Mte surfaces [26,27]. In our experiments, described above, a solution containing 10% SDS at 100 °C was used to remove the PrP^{Sc} from mineral surfaces. Changes in pH often alter interactions between clay surfaces and sorbed proteins [27,28]. Incubation of Mte-bound PrP^{Sc} in 100 mM phosphate buffer at pH 2.5 or 11.5, proton activities substantially higher and lower than the reported isoelectric points for PrP^{Sc} [29], failed to release the protein (Figure 3B). Likewise, increases in ionic strength (0.1 M or 1 M NaCl) failed to remove detectable PrP^{Sc} from Mte (Figure 3C). Strong chaotropic agents can be effective in desorbing proteins from soil minerals by disrupting hydrogen bonds [26]; however, neither 8 M urea nor 8 M guanidine released detectable amounts of PrP^{Sc} from Mte (Figure 3D). Our data indicate the interaction between PrP^{Sc} and Mte is strong and of high affinity.

PrP^{Sc} Bound to Mte Remains Infectious

Sorption of proteins to soil particles often results in structural rearrangements that cause loss or diminution of function [25,27,30]. If binding to Mte surfaces results in (partial) unfolding of PrP^{Sc}, a reduction or loss of infectivity would be expected, as denaturation renders the protein non-infectious [31]. We therefore tested whether PrP^{Sc} adsorbed to Mte remained infectious by intracerebrally inoculating hamsters with Mte-PrP^{Sc} complexes (Table 2). The time to onset of clinical symptoms after inoculation provides a measure of infectivity [32]. Hamsters inoculated with Mte-PrP^{Sc} exhibited clinical symptoms of scrapie 93 dpi. To control for any unbound prion protein that may have cosedimented with Mte particles, mineral-free PrP^{Sc} suspensions were processed in the same manner as in sorption experiments. The sedimented fraction of these control samples (mock pellets) showed substantially less infectivity than Mte-PrP^{Sc} pellets with a mean incubation period of 178 d, 105 d longer than Mte-PrP^{Sc} pellets. Hamsters inoculated with supernatants from these control samples (mock supernatants) showed clinical symptoms 103 dpi. Animals intracerebrally inoculated with Mte alone and uninoculated animals did not exhibit TSE symptoms during the course of the experiment (200 d).

Table 2. Prions Adsorbed to Montmorillonite Clay Retain Infectivity

Inoculum	Positive Animals/ Total Animals	Onset of Clinical Symptoms (dpi)[a]
None	0/8	>200[b]
Mte (no PrP^{Sc})	0/8	>200[b]
Mte-PrP^{Sc} complex	10/10[c]	93 ± 4[d]
Mock supernatant[e] (no Mte)	8/8	103 ± 0[d]
Mock pellet[e] (no Mte)	8/8	178 ± 21[d]

[a]Mean dpi ± SD to the onset of clinical symptoms of TSE infection.
[b]None of the animals showed clinical symptoms of TSE infection or had protease-resistant PrP accumulation at the termination of the experiment at 200 dpi.
[c]Although 12 animals were inoculated, two non-TSE intercurrent deaths occurred at 8 dpi.
[d]Brains of infected animals were positive for protease-resistant PrP.
[e]Mock supernatant and mock pellet samples were generated by adding clarified PrP^{Sc} (~0.2 μg) to buffer in the absence of soil minerals and processing identically to samples containing Mte.
DOI: 10.1371/journal.ppat.0020032.t002

Whole Soils Bind PrP^{Sc}

To examine the extent of prion protein binding by whole soils, we conducted PrP^{Sc} sorption experiments with four soils differing in texture and mineralogy (Table S2). When equal masses of soil (0.5 μg) were used, all soils bound PrP^{Sc} to a similar extent (Figure 4); no detectable PrP^{Sc} remained in the supernatant at the level of protein used in this experiment. Prion protein desorbed from the soils did not appear cleaved. Several nonmutually exclusive factors may have contributed to this finding, including (1) relatively small amounts of Mte in some samples, (2) occlusion of Mte cleavage sites by metal oxide and/or natural organic matter coatings, and (3) competition among the various sorption domains (both inorganic and organic) for PrP^{Sc}, limiting interaction with Mte. The amount of immunoreactive PrP^{Sc} recovered from each soil differed slightly; for example, the immunoreactive protein desorbed from the Elliot soil was less than that from the Boardman soil. This may have been due to stronger interaction of PrP^{Sc} with the Elliot soil than with the Boardman soil, leading to incomplete extraction, consistent with the larger fraction of clay-sized particles in the Elliot soil (Table S2).

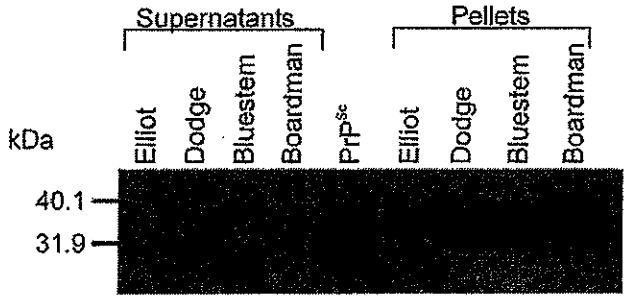

Figure 4. Whole Soils Bind PrP^{Sc}
Elliot, Dodge, Bluestem, and Boardman soils bound PrP^{Sc} (pelleted soils). No immunoreactivity (i.e., no unbound PrP^{Sc}) was detected in the supernatants. Immunoblot employed mAb 3F4.
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Discussion

Environmental transmission of prion diseases has been noted for decades [7,8,14]. In this study, we provide evidence indicating that soil and soil minerals serve as a reservoir of TSE infectivity. While extrapolation of in vitro studies to the environment must be made with caution, our findings suggest that PrP^{Sc} released from diseased animals may be sequestered near the soil surface, maintaining the TSE agent in an environmental medium with which livestock and wildlife come in contact. Our experiments demonstrate that Mte-bound PrP^{Sc} remains infectious and suggest that soil may harbor more TSE agent than previously assumed on the basis of water extraction of prions from garden soil [13].

Our results demonstrate that all soil mineral surfaces examined bound PrP^{Sc} and that Mte and quartz have larger specific binding capacities for PrP^{Sc} than does Kte (Figure 1). Although not relevant to TSE transmission, nonglycosylated, recombinant PrP^{C} has been shown to bind to Mte [33]. Interestingly, the N terminus of PrP^{Sc} desorbed from Mte was truncated (Figures 1A and 2). While Mte is known to catalyze several reactions, including the deamination of free glutamine and aspartic acid [34] and the polymerization of RNA into oligomers [35], protease activity has not been noted previously. The interaction between Mte and PrP^{Sc} is remarkably avid, as the only extractant used in this study that effected desorption was a solution containing 10% SDS at 100 °C (Figure 3B–3D). Prion protein appears unlikely to readily desorb from Mte in the environment. The propensity for PrP^{Sc} to tenaciously bind to Mte could be exploited in landfills to isolate prion-infected materials and prevent migration of the infectious agent.

The observation that prions remained infectious when bound to Mte is intriguing in light of the results of the desorption experiments; PrP^{Sc} adsorbed to Mte was extremely difficult to remove. Current mechanistic models for conversion of PrP^{C} to the pathological form require direct PrP^{C}–PrP^{Sc} interaction [36]. The brain is unlikely to possess microenvironments capable of extracting significant amounts of PrP^{Sc} from clay surfaces. The 10-d increase in incubation period for Mte-adsorbed PrP^{Sc} relative to clay-free controls (mock supernatant) was statistically significant ($p < 0.05$) and would correspond to approximately a 1-log increase in infectivity [32]. This result suggests that PrP^{Sc}-Mte complexes are inherently more infectious than the unbound protein and/or adsorption to Mte reduces clearance from the brain. We consider it likely that PrP^{Sc} adsorbed to Mte surfaces was available to convert PrP^{C} in the brain to the pathological isoform. Our findings are reminiscent of reports in which metal wires exposed to scrapie agent harbored significant infectious agent despite attempts to remove attached PrP^{Sc} [37,38].

The infectivity of soil- and soil mineral-sorbed PrP^{Sc} following oral exposure warrants investigation. The binding of PrP^{Sc} to soil particles could reduce oral bioavailability such that soil serves as a sink rather than a reservoir for infectivity. Conversely, association with mineral particles may protect the agent from degradation in the gastrointestinal tract, possibly enhancing transmission [39]. For example, bovine rotaviruses and coronaviruses retain infectivity via the oral route when bound to clay minerals [40]. While desorption of the protein from soil particles is more likely to occur in the

gut than in the brain, removal of PrP^{Sc} from mineral particles may not be necessary to initiate infection.

In conclusion, soil and soil minerals have the potential to bind PrP^{Sc} and maintain infectivity. These findings will serve as the basis for further study on the interaction of PrP^{Sc} with other soil components (humic substances, quartz, and other minerals), the stability of soil-bound PrP^{Sc} under typical environmental conditions (UV light, freeze-thaw cycles) and the effect of soil microorganisms and extracellular enzymes on protein integrity. Our current results suggest that sorption of PrP^{Sc} to clay minerals may limit its migration through the soil column. Maintenance of prion infectivity at the soil surface may contribute to the propagation of CWD and scrapie epizootics and enhance the likelihood of interspecies transmission of these diseases.

Materials and Methods

Preparation of soil minerals and soils. Montmorillonite (SWy-2) and kaolinite (KGa-1b) clays, obtained from the Clay Minerals Society Source Clays Repository (West Lafayette, Indiana, United States), were size-fractionated by wet sedimentation to obtain particles with d_h = 0.5–2 μm and saturated with sodium. These reference clay samples were extensively characterized previously [23,41]. Fine quartz sand (d_h = 125–250 μm) and SiO_2 microparticles (d_h = 1–5 μm; 99% purity) were obtained from Sigma (St. Louis, Missouri, United States). The fine quartz sand was soaked for 24 h in 12 N HCl to remove impurities. X-ray diffraction analysis and infrared photoacoustic spectroscopy indicated that the SiO_2 microparticles were composed of quartz.

We examined PrP^{Sc} sorption to four soils (Table S2). The Elliot soil was a silty clay loam purchased from the International Humic Substances Society (St. Paul, Minnesota, United States). Organically amended Dodge soil (sandy clay loam) was obtained from a glaciated upland area in Madison, Wisconsin. The Bluestem soil was a sandy clay loam collected from a fluvial deposit in Cedar Rapids, Iowa. The Boardman soil was a silt loam taken from an eolian deposit in Boardman, Oregon. Characteristics of these soils are presented in Table S2.

Source of PrP^{Sc}. Syrian hamsters (cared for according to all institutional animal care and handling protocols of the University of Wisconsin, Madison) were experimentally infected with the Hyper strain of hamster-adapted transmissible mink encephalopathy agent. PrP^{Sc} was purified to a P_4 pellet from brains of infected hamsters by a modification of the procedure described by Bolton et al. [42,43]. The P_4 pellet prepared from four brains was resuspended in 1 ml of 10 mM Tris (pH 7.4) with 130 mM NaCl. For experiments employing PK-treated PrP^{Sc}, 20% brain homogenate was treated with 50 μg ml^{-1} of *proteinase K for 30 min at 37 °C. After blocking PK activity with 5* mM phenylmethylsulfonyl fluoride, purification was performed as above.

Batch sorption experiments. Larger prion aggregates were removed from purified PrP^{Sc} by collecting supernatants from two sequential 5-min centrifugations at 800 *g* (clarification step). Clarified PrP^{Sc} (~0.2 μg) was added to 500 μg of Mte or fine quartz sand, 1,500 μg of Kte, or 3.2 mg of quartz microparticles in 10 mM NaCl buffered to pH 7.0 with 10 mM 3-*N*-morpholinopropanesulfonic acid (MOPS) (500 μl final volume). In some cases, Mte experiments were conducted in unbuffered 10 mM NaCl. Sorption experiments with Mte performed in buffered and unbuffered 10 mM NaCl yielded comparable results. Experiments with Mte, Kte, and quartz microparticles each employed equivalent (external) mineral surface areas. In sorption experiments with whole soil samples, ~2 μg of clarified PrP^{Sc} was added to 5-ml suspensions of each soil (5 mg) in 5 mM $CaCl_2$. Samples were rotated at ambient temperature for 2 h or an indicated time period. Sorption appeared complete within 2 *h*, as longer incubation times did not result in changes in levels of bound protein.

Each PrP^{Sc}-mineral suspension and a 500-μl aliquot of each PrP^{Sc}-soil suspension was placed over a 750 mM sucrose cushion prepared in a solution of the same composition as the background solution in the sorption experiment, and centrifuged at 800 *g* for 7 min to sediment mineral or soil particles and adsorbed PrP^{Sc}. A sucrose cushion was found necessary to prevent a fraction of unbound PrP^{Sc} from *sedimenting during centrifugation. Clarified* PrP^{Sc} *did not* sediment through the sucrose cushion (Figure S1).

Unbound PrP^{Sc} remaining in the supernatant was precipitated with four volumes of cold methanol and resuspended in SDS-PAGE sample buffer (100 mM Tris [pH 8.0], 10% SDS, 7.5 mM EDTA, 100 mM dithiothreitol, and 30% glycerol). PrP^{Sc} was extracted from pelleted mineral particles with SDS-PAGE sample buffer at 100 °C for 10 min. The same procedure was followed for PrP^{Sc}-soil suspensions. To determine mineral adsorption capacities for prion protein, varying volumes of clarified PrP^{Sc} preparation were added to a 1:100 dilution of each mineral suspension. All adsorption experiments were repeated at least three times.

For BH sorption experiments, 10% BH was clarified by collecting supernatants from two sequential 5-min centrifugations at 800 *g*. Aliquots (10 or 30 μl) of clarified BH were rotated with Mte in 10 mM NaCl at ambient temperature for 2 h; complexes of Mte and BH constituents were then sedimented through a sucrose cushion and processed as described in the preceding paragraphs.

All samples prepared for SDS-PAGE were separated on 4%–20% precast gels (BioRad, Hercules, California, United States) under reducing conditions. Proteins were transferred to polyvinyl difluoride membranes and immunoblotted with mAb 3F4 (1:40,000 dilution), R20 N-terminal pAb (1:10,000 dilution), Rab 9 pool 2 full-length PrP pAb (1:10,000 dilution), or anti-20*S* proteosome subunit C2 pAb (1 μg ml^{-1}; A.G. Scientific, San Diego, California, United States). Detection was achieved with an HRP-conjugated goat anti-mouse immunoglobulin G (IgG) (BioRad) for mAb 3F4 and an HRP-conjugated goat anti-rabbit IgG (BioRad) for all pAbs.

X-ray diffraction analysis. PrP^{Sc} preparation (10 μg) was added to 50 μg of Mte in 10 mM NaCl (final volume of 0.5 ml). Samples were rotated at ambient temperature for 2 h and centrifuged at 16,100 *g* for 7 min. After centrifugation, the bulk of the supernatant was removed, leaving a small amount of solution above the clay pellet. The clay was resuspended in the remaining supernatant, and the slurry was placed on silica wafer slides and stored in a desiccator for over 12 h. The basal d_{001} spacings of near homoionic Na^+-SWy-2 before and after adsorption of PrP^{Sc} were determined by X-ray diffraction on a Scintag PAD V diffractometer (Cupertino, California, United States) using CuKα radiation and continuous scanning from 3° to 15° 2θ with a step size of 0.02° and a dwell time of 2 s.

Extraction experiments. PrP^{Sc} adsorbed to Mte was incubated for 30 min at room temperature in 8 M urea or 8 M guanidine HCl (50 μl per pellet), 0.1 or 1 M NaCl (25 μl per pellet), or 100 mM sodium phosphate (pH 2.5 or 11.5; 25 μl per pellet). Primary extractions with these solutions were followed by secondary extractions with SDS-PAGE sample buffer at 100 °C to assess the efficacy of the primary extraction. Urea and guanidine primary extracts were dialyzed against double distilled water for 2 h (nominal molecular weight cutoff, 12–14 kDa; Fisher Scientific, Pittsburgh, Pennsylvania, United States) prior to SDS-PAGE analysis.

Infectivity bioassay. PrP^{Sc}-Mte pellets prepared as above were resuspended in pH 7.4 PBS (50 μl per pellet) and intracerebrally inoculated into male, weanling Syrian hamsters (Harlan, Indianapolis, Indiana, United States). Equivalent amounts of PrP^{Sc} starting material or Mte without PrP^{Sc} were inoculated into control animals. Hamsters were monitored every 3 d for the onset of clinical symptoms [32,44]. Brains from clinically positive hamsters and uninfected controls were analyzed for protease-resistant PrP by immunoblotting.

Supporting Information

Figure S1. Sucrose Cushion Prevented Sedimentation of Unbound PrP^{Sc} under Conditions Necessary to Pellet Soil Minerals

A substantial amount of unbound PrP^{Sc} pelleted when centrifuged under conditions required to remove Na^+-Mte from suspension, but was prevented from sedimenting by a sucrose cushion. Sucrose cushions were therefore employed in batch sorption experiments to prevent sedimentation of unbound PrP^{Sc}. Results from representative mock adsorption experiments are shown. PrP^{Sc} was rotated in a solution of 10 mM NaCl in the absence of soil minerals for 2 h and was either placed above a 750 mM sucrose cushion and centrifuged (two right lanes), or centrifuged without a sucrose cushion (two left lanes). Supernatants (Sup) and pellets (Pel) were analyzed by immunoblotting with mAb 3F4.

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Table S1. Characteristics of Minerals Used in PrP^{Sc} Sorption Experiments

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Table S2. Characteristics of Soils Used in PrP^{Sc} Sorption Experiments

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Accession Numbers

The GenBank (http://www.ncbi.nlm.nih.gov/) accession number for PrP^{Sc} is MI4054.

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Author contributions. CJJ, DM, JMA, and JAP conceived and designed the experiments. CJJ, KEP, and PTS performed the experiments. CJJ, KEP, PTS, DM, JMA, and JAP analyzed the data. JMA and JAP contributed reagents/materials/analysis tools. CJJ, DM, JMA, and JAP wrote the paper.

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Competing interests. The authors have declared that no competing interests exist. ■

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医薬品 研究報告 調査報告書

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研究報告の概要

○慢性消耗病(CWD)に感染したシカの骨格筋におけるプリオン

シカやエルクにおけるCWDの流行は広い地域に広がっており、ウシ海綿状脳症が変異型クロイツフェルト・ヤコブ病としてヒトへ種間伝播したことと同様に、CWDが人畜共通感染を起こすのではないかという懸念が起こっている。食肉の摂取が最も可能性の高い暴露の経路であるため、感染したシカ科の動物の骨格筋に感染性プリオンが含まれているかを明らかにすることが重要である。シカプリオン蛋白を発現したトランスジェニックマウスにおける動物実験で、CWDに感染したシカの骨格筋に感染性プリオンが存在することが明らかになり、CWDに感染したシカ肉を摂取あるいは取り扱う人はプリオンへの暴露のリスクがあることが示された。

報告企業の意見	今後の対応
CWDに感染したシカの骨格筋に感染性プリオンが存在することが明らかになり、CWDに感染したシカ肉を摂取あるいは取り扱う人はプリオンへの暴露のリスクがあることが示されたとの報告である。	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

解凍赤血球濃厚液「日赤」
照射解凍赤血球濃厚液「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

(17)

Prions in Skeletal Muscles of Deer with Chronic Wasting Disease

Rachel C. Angers,[1*] Shawn R. Browning,[1*†] Tanya S. Seward,[2] Christina J. Sigurdson,[4‡] Michael W. Miller,[5] Edward A. Hoover,[4] Glenn C. Telling[1,2,3§]

Prions are transmissible proteinaceous agents of mammals that cause fatal neurodegenerative diseases of the central nervous system (CNS). The presence of infectivity in skeletal muscle of experimentally infected mice raised the possibility that dietary exposure to prions might occur through meat consumption (*1*). Chronic wasting disease (CWD), an enigmatic and contagious prion disease of North American cervids, is of particular concern. The emergence of CWD in an increasingly wide geographic area and the interspecies transmission of bovine spongiform encephalopathy (BSE) to humans as variant Creutzfeldt Jakob disease (vCJD) have raised concerns about zoonotic transmission of CWD.

To test whether skeletal muscle of diseased cervids contained prion infectivity, Tg(CerPrP) mice (*2*) expressing cervid prion protein (CerPrP) were inoculated intracerebrally with extracts prepared from the semitendinosus/semimembranosus muscle group of CWD-affected mule deer or from CWD-negative deer. The availability of CNS materials also allowed for direct comparisons of prion infectivity in skeletal muscle and brain. All skeletal muscle extracts from CWD-affected deer induced progressive neurological dysfunction in Tg(CerPrP) mice, with mean incubation times ranging between 360 and ~490 days, whereas the incubation times of prions from the CNS ranged from ~230 to 280 days (Table 1). For each inoculation group, the diagnosis of prion disease was confirmed by the presence of disease-associated, protease-resistant PrP (PrP^{Sc}) in the brains of multiple infected Tg(CerPrP) mice [see (*3*) for examples]. In contrast, skeletal muscle and brain material from CWD-negative deer failed to induce disease in Tg(CerPrP) mice (Table 1), and PrP^{Sc} was not detected in the brains of asymptomatic mice as late as 523 days after inoculation (*3*).

Our results show that skeletal muscle as well as CNS tissue of deer with CWD contains infectious prions. Similar analyses of skeletal muscle from BSE-affected cattle did not reveal high levels of prion infectivity (*4*). It will be important to assess the cellular location of PrP^{Sc} in muscle. Although PrP^{Sc} has been detected in muscles of scrapie-affected sheep (*5*), previous studies failed to detect PrP^{Sc} by immunohistochemical analysis of skeletal muscle from deer with natural or experimental CWD (*6*, *7*). Because the time of disease onset is inversely proportional to prion dose (*8*), the longer incubation times of prions from skeletal muscle extracts compared with those from matched brain samples indicated that prion titers were lower in muscle than in the CNS, where infectivity titers are known to reach high levels. Although possible effects of CWD strains or strain mixtures on these incubation times cannot be excluded, the variable 360- to ~490-day incubation times suggested a range of prion titers in skeletal muscles of CWD-affected deer. Muscle prion titers at the high end of the range produced the fastest incubation times, which were ~30% longer than the incubation times of prions from the CNS of the same animal. Because all mice in each inoculation group developed disease, prion titers in muscle samples producing the longest incubation times were higher than the end point of the bioassay, defined as the infectious dose at which half the inoculated mice develop disease. Although the risk of exposure to CWD infectivity after consumption of prions in muscle is mitigated by relatively inefficient prion transmission via the oral route (*9*), our results show that semitendinosus/semimembranosus muscle, which is likely to be consumed by humans, is a major source of prion infectivity. Humans consuming or handling meat from CWD-infected deer are therefore at risk to prion exposure.

Table 1. Incubation times after inoculation of Tg(CerPrP) mice with prions from skeletal muscle and brain samples of CWD-affected deer. PBS, phosphate buffered saline.

Inocula	Incubation time, mean days ± SEM (n/n_0)*	
	Skeletal muscle	Brain
	CWD-affected deer	
H92	360 ± 2 (6/6)	283 ± 7 (6/6)
33968	367 ± 9 (8/8)	278 ± 11 (6/6)
5941	427 ± 18 (7/7)	
D10	483 ± 8 (8/8)	231 ± 17 (7/7)
D08	492 ± 4 (7/7)	
Averages	426	264
	Nondiseased deer	
FPS 6.98	>523 (0/6)	
FPS 9.98	>454 (0/7)	>454 (0/6)
None	>490 (0/6)	
PBS	>589 (0/5)	

*The number of mice developing prion disease (n) divided by the original number of inoculated mice (n_0) is shown in parentheses. Mice dying of intercurrent illnesses were excluded.

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Supporting Online Material

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Materials and Methods
Fig. S1

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別紙様式第2-1

No. 19

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. 3. 5	該当なし	

一般的名称	解凍人赤血球濃厚液	研究報告の公表状況		公表国
販売名(企業名)	解凍赤血球濃厚液「日赤」(日本赤十字社) 照射解凍赤血球濃厚液「日赤」(日本赤十字社)		Sundayherald. 2006 Mar 5. Available from: URL: http://www.sundayherald.com/54442	英国

研究報告の概要

○CJD専門家が「ヒツジのBSE」を警告

非定型スクレイピーと呼ばれるヒツジやヤギの脳疾患は、BSEに似ており、2003年に流行が始まった。今では英国中で82,000頭ものヒツジが罹患していると見積もられており、他の欧州諸国でも症例が報告されている。

現在、生後18ヶ月を越えたヒツジ20,000頭に対しては毎年TSEの検査を行っており、今までに非定型スクレイピー108例が発見された。しかしvCJD専門家のDr. Stephen Deallerは、この疾患がどの程度まで広がっているかを把握するために、もっと若い動物に対して緊急に検査を行うよう求めている。彼は、農業への影響を懸念して大規模な検査が行えないのではないかと示唆している。Deallerは、政府が人への感染の危険があると認める6年前に、共同研究者とともにBSEに関して警告を発している。彼の調査要求は他の消費者団体からも支持されている。

現在の消費者保護規定では、BSEの感染性が高いと考えられる動物の部位(脳など)は流通工程から取り除かれる。しかし、非定型スクレイピーが他の部位から感染するかどうかは不明である。

政府に対して助言する独立科学委員会は人や動物の健康への影響について確実なリスク分析をするにはデータが不十分であると話した。海綿状脳症諮問委員会は、より多くの情報を提供するために綿密な調査が重要でありすぐに行うべきだと述べた。

食品基準庁(FSA)は今後この問題を検討する予定であり、「理論上は危険」があるとしながらも、消費者にヒツジやヤギの肉を食べないよう推奨することはしていない。

微生物学会の会長で食品基準の専門家であるHugh Penningtonは非定型スクレイピーが人に害をもたらすとは言えないと話している。「人間は200年スクレイピーのヒツジを食べてきたが、誰も感染していない」

報告企業の意見	今後の対応
非定型スクレイピーと呼ばれるヒツジやヤギの脳疾患に関して、専門家が緊急に検査を行うよう求めているとの報告である。	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

解凍赤血球濃厚液「日赤」
照射解凍赤血球濃厚液「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

Sunday Herald – 05 March 2006

CJD expert warns of 'BSE in sheep'

Scientist who told of threat to humans from cattle calls for urgent study to find out how many animals have new disease

By Judith Duffy, Health Correpondent

A leading vCJD expert who sounded the alarm on BSE has called for the government to "take action right now" over fears that a recently discovered brain disease in sheep and goats could pose a risk to human health.

The disease, known as atypical scrapie, is similar to BSE in cattle and first emerged in 2003. It is now estimated that as many as 82,000 sheep could be infected in the UK and cases have been reported in other European countries.

The Food Standards Agency (FSA), has admitted there is a "theoretical risk" but it is not recommending that consumers stop eating sheep or goat meat.

However, vCJD expert Dr Stephen Dealler has demanded an immediate investigation to determine the extent of the disease. Lancaster-based microbiologist Dealler and his colleague Professor Richard Lacey warned the government about the dangers of BSE in cattle six year before ministers conceded there was a risk to humans.

"The worry is, of course, that atypical scrapie will be infectious to humans, but we don't know," Dealler said.

"All I can say at the moment is that with atypical scrapie, let's wait and see - but should we, in this wait-and-see period, be taking more aggressive action?

"Lots of people are saying we shouldn't just stand here and wait, lots of people are saying take action right now."

Under current regulations, 20,000 sheep in the UK over 18 months old are tested annually for brain diseases known as transmissible spongiform encephalopathies (TSE). These include atypical scrapie as well as the more common form of scrapie and BSE.

To date, a total of 108 cases of atypical scrapie have been detected via this testing programme. But Dealler called for further testing to be urgently carried out, particularly in younger animals, to determine exactly how widespread it is.

"At the moment, without the data on how much disease is out there, it is difficult to know what to do and how fast to act," he said. "That is why I say we need a survey right now.

"What they could certainly do is to do surveys and take so many sheep, test them when they are being slaughtered, and then see what proportion of those is atypical form.

"You can find BSE in the brains of cows long, long before they showed any symptoms at all and this will almost certainly be true with scrapie as well."

He suggested that concerns about the impact on farming were likely to be hindering an expansion in testing.

Current controls to protect consumers mean that parts of animals most likely to carry BSE infectivity - such as brains - are removed from sheep and cattle before entering the food chain. But it is uncertain if atypical scrapie could be carried in other tissue.

Dealler's calls for an investigation have been backed by consumer groups.

Sue Davies, Which? chief policy adviser, said: "We need urgent answers as to the many uncertainties surrounding this finding as quickly as possible so that there is a better understanding of whether there are any human health implications and, if so, whether existing control measures are adequate."

An independent scientific committee that advises the government said last week there is "insufficient data, as yet, to make reliable risk assessments for human health or animal health and welfare". In a statement, the Spongiform Encephalopathy Advisory Committee (Seac) also concluded that rigorous studies are "critical and urgent" to provide more information.

The FSA is due to initially examine the issue at a board meeting on Thursday . Possible options for precautionary risk reduction measures will be then discussed next month. An FSA spokeswoman said she could not pre-empt discussions by suggesting what - if any - measures might be taken.

"We can't rule out any theoretical risk, but we won't be changing our advice at this stage ," she said. "Based on the information we have, we are not recommending people change their eating habits on sheep or goats."

Professor Hugh Pennington, president of the Society for General Microbiology and an expert on food standards, said current evidence did not suggest atypical scrapie was a threat to humans.

He added: "The big question is: what implications does it have for human health? As far as we know, there are none basically, but of course we have to keep on doing research on this.

"One certain thing is that we have been eating scrapied sheep for 200 years and nobody has come to any harm."

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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	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.guardian.co.uk/frontpage/story/0,,1765531,00.html	公表国：英国	
販売名（企業名）	－					

研究報告の概要

英国政府は 1990 年代に輸出した英国製汚染血漿分画製剤により、患者が vCJD を発症するリスクにさらされていると 14 ヵ国（ブラジル、トルコ、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、ベルギー、モロッコ、エジプト、フランス、オランダおよびイスラエル）に警告した。
問題は血漿分画製剤が数千人の血液から製造されいていることであり、科学者は未発症の感染者の供血によって引き起こされる「第二波」の災害を懸念している。
血液を介した感染リスクは 2003 年 12 月までは仮説に過ぎなかったが、その後輸血を介して感染した英国人患者が出現し、さらに 2 例が見いだされていることから、保健当局は国立企業の Bio Products Laboratory（BPL）により国外に輸出された血液製剤を再調査しなければならなくなった。
保健保護局は、輸出量や危険性を勘案し、最も危険性の高いブラジルとトルコ、それより危険性は低いが予防措置を講じる必要がある 6 ヵ国（ブルネイ、アラブ首長国連邦、インド、ヨルダン、オマーンおよびシンガポール）へは、予防措置（患者を追跡し、血液や臓器を提供しないよう通知すること、治療を必要とする場合は医師や歯科医に知らせるよう通知すること）を講じるように保健省に勧告した。危険性の低いベルギー、モロッコ、エジプトと、血液製剤を製造できるフランス、オランダおよびイスラエルについては自ら評価するよう勧告した。
BPL を管理する NHS 血液・移植当局は「現在のところ血漿分画製剤と関連づけられた vCJD 症例はない。」としている。

報告企業の意見	今後の対応
vCJD 多発国である英国が 14 ヵ国に血漿分画製剤を輸出していたので、該当国に対し警告を発したとの報告である。報告中で NHS は「現在のところ血漿分画製剤と関連づけられた vCJD 症例はない。」と述べている。 なお、当社では英国より血漿分画製剤又はその原料血漿を輸入したことはない。	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。

使用上の注意記載状況・その他参考事項等

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。

theguardian

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.

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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

医薬品 研究報告 調査報告書

識別番号・報告回数		1	報告日	第一報入手日 2006 年 4 月 10 日	新医薬品等の区分	厚生労働省処理欄
一 般 的 名 称	別紙のとおり		研究報告の公表状況	Identification of a Novel Single-Stranded DNA Fragment Associated with Human Hepatitis J. Inf,,Dis. 15;193(8):1089-97. 2006	公表国 日本	
販 売 名（企 業 名）	別紙のとおり					

研究報告の概要

（問題点：原因不明の急性肝炎発症患者の血液から、A型からE型ではない未知のDNA配列を持つ「NV－F」感染症が確認された。）

原因不明の急性肝炎を発症した患者の血液から、未知のDNA配列「NV-F」を発見。A型からE型までの肝炎ウイルスが検出されない、原因不明の肝炎患者 69 人中の 17 人（24.6%）で、NV-F が検出された。

17 人のうち 1 人は劇症肝炎で、NV-F は発症から約 10 日間、血中に現れ、症状の回復につれて消えた。この患者の肝細胞からは、NV-F が作り出した抗原が検出され、NV-F が肝臓で増殖したことをうかがわせた。

NV-F は、B 型や C 型と同じ経路で感染しやすいウイルスの DNA と推測される。

使用上の注意記載状況・その他参考事項等

記載なし

報告企業の意見	今後の対応
別紙のとおり	現時点においては、特段の対応は不要と考えるが、今後とも関連情報の収集に努め、本剤の安全性の確保を図っていきたい。

MAJOR ARTICLE

Identification of a Novel Single-Stranded DNA Fragment Associated with Human Hepatitis

Chau-Ting Yeh, Mei-Lin Tsao, Ying-Chun Lin, and I-Chu Tseng

Liver Research Unit, Chang Gung Medical Center, Taipei, Taiwan

By performing nonspecific polymerase chain reaction followed by elimination of chromosome-derived sequences, foreign DNA fragments were obtained from the serum of a patient with non–A–E hepatitis. One of the sequences, named NV-F, contained a partial open reading frame and was detected in 17 (24.6%) of 69 patients with non–A–E hepatitis, including 1 with fulminant hepatitis (vs. in 5 [2.8%] of 180 healthy individuals). A peptide was synthesized accordingly, to detect serum anti–NV-F antibody, which was found in 49 (75.4%) of 65 patients positive for NV-F. This DNA fragment was sensitive to S1 nuclease digestion. Cesium chloride gradient analysis revealed that the NV-F–associated particles had buoyant densities of 1.33–1.39 and 1.22–1.25 g/mL. Immunofluorescence analysis revealed that the novel antigen was present in the hepatocytes of patients infected with NV-F. In conclusion, we have identified a novel single-stranded DNA fragment derived from a virus-like agent associated with human hepatitis.

Previously, when diagnostic tests for the detection of hepatitis A and B viruses (HAV and HBV) were globally available, it had been recognized that a significant proportion of patients with acute and chronic hepatitis were not infected with either virus, and the diseases were referred to as "non-A, non-B hepatitis" [1]. Owing to technological advances in molecular biology, hepatitis C and hepatitis E viruses (HCV and HEV) were subsequently discovered to be the major causes of parenteral and enteric non-A, non-B hepatitis, respectively [2]. Despite this significant progress, the etiology of acute and chronic hepatitis in a substantial number of patients remains unknown. In our previous studies, we found that 15.9% of hospital inpatients with acute hepatitis had non–A–E hepatitis [3]. Additionally, 9.7% of patients with fulminant hepatitis had non–A–E hepatitis [4]. Another study indicated that no definite etiology could be found in 4.9% of patients with chronic hepatitis or cirrhosis; these cases were termed "cryptogenic" [5]. Approximately half of these patients had received transfusions, which supported a virological etiology. Furthermore, enhanced HLA expression in liver samples from patients with chronic non–A–C hepatitis has been reported, which also supports a virological etiology [6]. Therapeutic trials using interferon-α to treat chronic non–A–C hepatitis have consistently resulted in an ~50% response rate, indicating a viral pathogen [7]. Inspired by these observations, scientists struggled to unearth the theoretically existing hepatitis viruses. As a result, several new viruses, including GB virus type C (GBV-C) [8], TTV [9], and SEN virus [10], were discovered. However, epidemiological data failed to confirm a causative role for these viruses in hepatitis. In addition, a high percentage of individuals infected by these viruses were found to be healthy carriers. Furthermore, in some studies, it was argued that GBV-C was not, in fact, a hepatotropic virus [8].

In the present article, we describe a novel agent associated with human hepatitis. Epidemiological data suggest that it is highly associated with non–A–E hepatitis. Biochemical evidence indicates that it is hepatotropic. Additionally, it was detected in a patient with fulminant non–A–E hepatitis.

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The Journal of Infectious Diseases 2006; 193:1089–97

0022-1899/2006/19308-0006$15.00

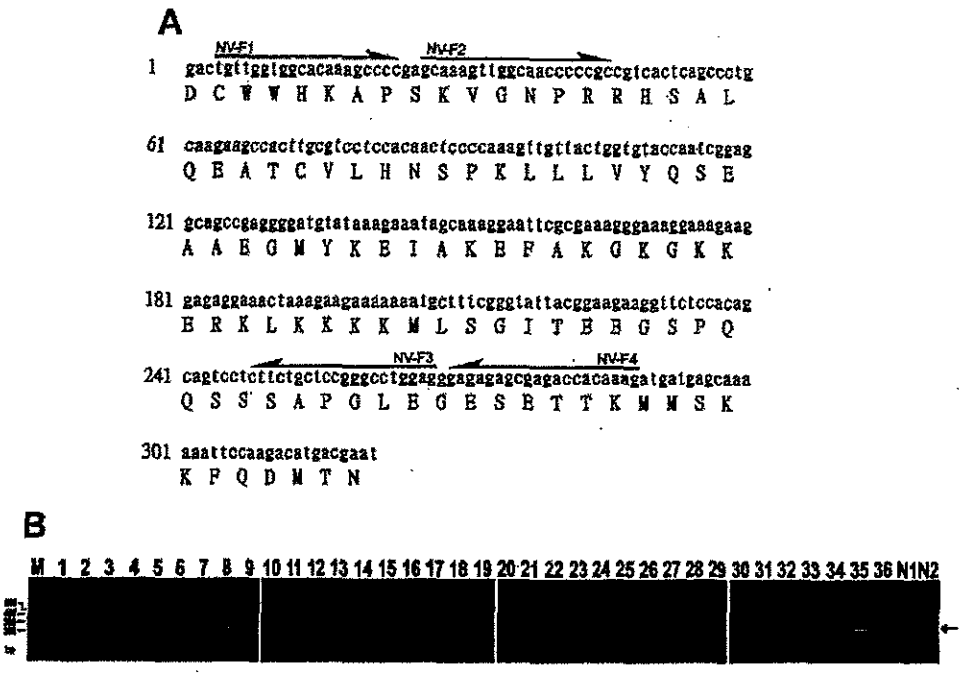

Figure 1. Identification of a foreign sequence in patients with non–A–E hepatitis. *A*, Nucleotide sequence of the NV-F DNA fragment and conceptual translation of the putative partial reading frame. The positions of 4 primers (NV-F1 to NV-F4) used for polymerase chain reaction (PCR) detection of NV-F are marked with arrows. *B*, Serum samples from patients with non–A–E hepatitis *(lanes 1–9)*, patients infected with hepatitis C virus *(lanes 10–19)*, patients infected with hepatitis B virus *(lanes 20–29)*, and healthy individuals *(lanes 30–36)* subjected to an NV-F detection assay. Only part of the results is shown here. M, molecular weight marker; N1, negative control (NV-F–negative serum sample); N2, negative control (pure water). The arrow indicates the PCR product of NV-F.

PATIENTS, MATERIALS, AND METHODS

Patients and samples. After informed consent was obtained, the remaining aliquots of serum samples submitted for biochemical tests in patients visiting Chang Gung Medical Center were collected for this study. Samples from 4 groups of patients were included for NV-F sequence detection: (1) 180 healthy subjects (from Health Examination Service, Chang Gung Medical Center) with normal alanine aminotansferase (ALT) levels who were negative for HBV surface antigen (HBsAg), anti-HCV antibody, and HEV RNA; (2) 150 patients with hepatitis B who were positive for HBsAg and negative for IgM class anti-HAV antibody, anti-HDV antibody, anti-HCV antibody, and HEV RNA; (3) 150 patients with hepatitis C who were negative for HBsAg and IgM anti-HAV antibody, positive for anti-HCV antibody, and negative for HEV RNA; and (4) 69 patients with non–A–E hepatitis with serum ALT levels elevated >2.5-fold who were negative for HBsAg, IgM anti-HAV antibody, IgM class antibody against HBV core antigen (HBc), anti-HCV antibody, HEV RNA, and HCV RNA. None of these patients were alcoholics, and no known hepatotoxic medicine had been taken. Patients with fatty liver were not excluded from this study. All patients were negative for autoimmune markers, including antinuclear antigen, anti–smooth muscle antigen, and anti-mitochondrial antigen. In addition, patients were all negative for other virological markers, including antibody for HIV, IgM class antibody for Epstein-Barr virus, and IgM class antibody for cytomegalovirus. After the polymerase chain reaction (PCR) assays for the NV-F sequence, adequate amounts of samples were still available for the detection of anti–NV-F antibody in 155 patients. After informed consent was obtained, liver biopsy samples from 2 patients (patients F and B) whose serum was positive for the NV-F sequence were subjected to immunofluorescence analysis.

Serological studies. HBsAg, IgM anti-HAV antibody, IgM anti-HBc antibody, and anti-HDV antibody were assayed using radioimmunoassay kits (Ausria-II, HAVAB-M, and anti-delta; Abbott Laboratories). Anti-HCV antibody was detected using an enzyme immunoassay kit (HCV-II; Abbott Laboratories). HCV RNA was detected by reverse transcription (RT) PCR assay (Amplicor HCV test; Roche Diagnostic Systems). HBV DNA was detected by Amplicor HBV Monitor Test (Roche Molecular Systems). The method of HEV RNA detection has been described elsewhere [3, 4].

Extraction of DNA or RNA, RT-PCR, and PCR. Total serum DNA was extracted using proteinase K digestion followed by phenol/chloroform extraction, as described in our previous publication [11]. Total serum RNA was extracted using TRI

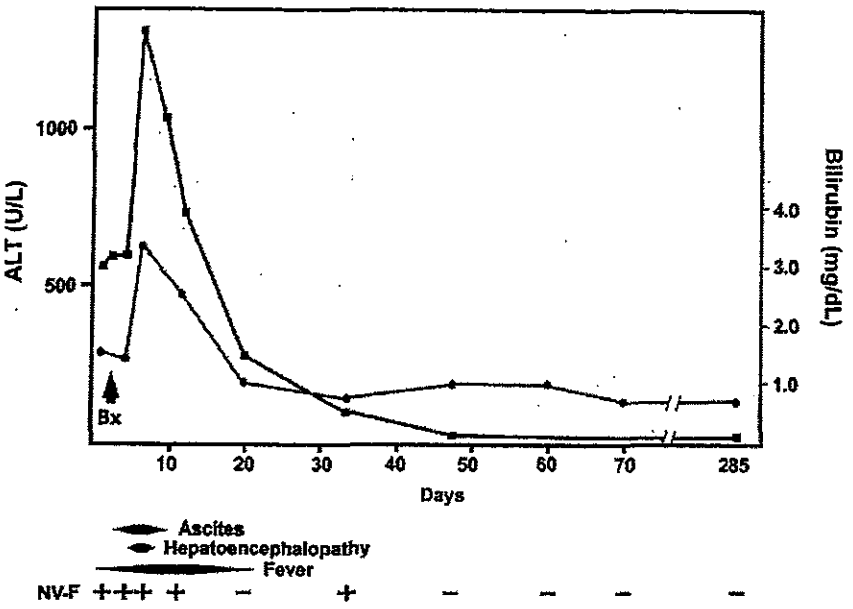

Figure 2. Clinical course in a patient with fulminant non-A–E hepatitis. Squares denote alanine aminotansferase (ALT) levels (U/L), and circles denote bilirubin levels (mg/dL). The periods of clinical symptoms are marked with solid bars, and "Bx" indicates the time of the liver biopsy. The NV-F sequence was detected by polymerase chain reaction in serial serum samples, and the results are indicated by a plus or a minus symbol.

reagent (Molecular Research Center), in accordance with the protocol provided by the manufacturer. RT was performed using random primers. The procedure for RT and PCR has been described elsewhere [12]. Three primers were engineered: P1, 5′-CCGCGG$(N)_4$-3′; P2, 5′-GAATTC$(N)_4$-3′; and P3, 5′-GCTTGCTCTGTCTC$(T)_{20}$-3′. Each of the 4 Ns in P1 and P2 was a mixture of A, T, C, and G in equal ratios. After extraction of the total serum DNA or RNA from patient L, PCR or RT-PCR was performed, using random hexamers for 25 cycles; the product was then amplified using any 2 of the P1–3 primers. The resulting products were cloned into a vector, pCR2.1-TOPO (Invitrogen). For PCR detection of *Escherichia coli* 16S ribosomal DNA, the following primers were used: 16SL, 5′-GTCTGGGAAACTGCCTGATG-3′ (nt 121–140) and 16SR, 5′-GCTTCTTCTGCGGGTAACGT-3′ (nt 500–481).

Elimination of clones derived from the human genome. To eliminate clones derived from the human genome, the clones were first lifted onto a nitrocellulose filter and hybridized with a mixture of probes generated from total liver RNA, as described in our previous publication [13]. Briefly, single-stranded probe was generated from cytoplasmic RNA extracted from normal human liver tissue. The tissue was minced into small pieces and lysed in a buffer containing 10 mmol/L Tris HCl (pH 7.2), 150 mmol/L NaCl, and 0.5% Nonidet P-40 (Sigma). After centrifugation at 1500 *g* for 5 min, the supernatant was used for RNA extraction. RT was performed using SuperScript II RNase H minus Reverse Transcriptase (Invitrogen), and oligo(dT) was used as the RT primer. One-third of the dTTP in the dNTP mixture was replaced by digoxigenin-11-dUTP (Boehringer Mannheim) to generate digoxigenin-labeled probes. The probes were mixed (molar ratio, 1:2) with oligo(dA) at 40°C for 1 h before hybridization. The hybridization signal was detected by use of a DIG Luminescent Detection Kit (Boehringer Mannheim). For each batch of hybridization, 1 ng of pCR2.1-TOPO without a cDNA insert was used as a negative control, and 1 pg of pCR2.1-TOPO containing a fragment of human albumin gene (Hs.184411) was used as a positive control. The negatively hybridized clones were considered to be of nonhuman origin.

Automatic sequencing. The nonhuman-origin clones were subjected to automatic DNA sequencing (CEQ 2000; Beckman Instruments). The sequence data were further searched against the National Center for Biotechnology Information (NCBI) human genome data bank (http://www.ncbi.nlm.nih.gov/genome/seq/HsBlast.html), to eliminate human sequence.

Development of anti–NV-F antibody. The putative partial coding sequence of NV-F, flanked by NV-F1 and NV-F4 primers, was inserted into a vector, pYES2/NT (Invitrogen Corporation), and was arranged in-frame with the upstream polyhistidine region and the Xpress epitope sequence. The coding region of the whole fusion protein was subsequently isolated by restriction enzyme digestion (*Hin*dIII to *Xba*I), blunt-ended, and inserted into the *Sma*I site of pBacPAK8 (Clontech Laboratories). The fusion protein was expressed using the BacPak Baculovirus Expression System (Clontech). It was purified by a Ni^{2+}-charged

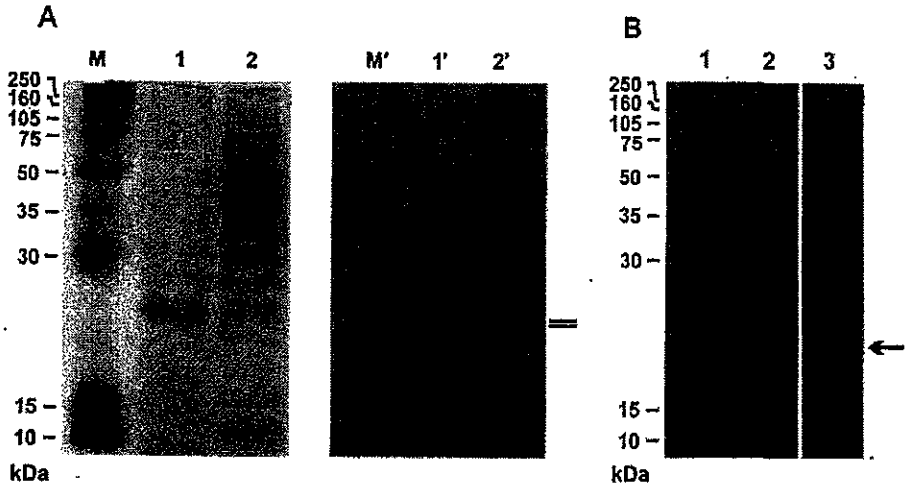

Figure 3. Generation of NV-F peptide and development of antibody against NV-F antigen. *A*, A fusion protein containing polyhistidine, Xpress epitope, and a peptide encoded by NV-F was expressed in insect cells. The protein extract was purified by affinity column and was analyzed by electrophoresis. The molecular weight marker (*M* and *M'*), purified protein (*lanes 1* and *1'*), and nonpurified cell lysate (*lanes 2* and *2'*) were visualized by either coomassie blue staining (*M*, *lanes 1* and *2*) or Western blot analysis using anti-Xpress antibody (*M'*, *lanes 1'* and *2'*). The purified protein was then used to develop a mouse polyclonal antibody against NV-F. *B*, The NV-F peptide alone (no fusion parts), subsequently expressed in insect cells. The cell lysate containing NV-F peptide *(lane 1)* and a mock control *(lane 2)* were analyzed by Western blot using the mouse anti–NV-F antibody. The cell lysate containing NV-F peptide was also analyzed, using a patient's serum that was positive for the NV-F sequence *(lane 3)*.

affinity column and was injected into a mouse for development of a polyclonal antibody. Alternatively, an initiation codon (ATG) was engineered in-frame with the putative coding sequence, and the resulting sequence was inserted into pBacPAK8, to express an NV-F peptide that did not contain any fusion parts. The primer used to generate the initiation codon (underlined) was 5′-ATGTGTTGGTGGCACAAAGCCC-3′.

Immunofluorescence analysis. Fragments of liver specimens were snap frozen in isopentane cooled with liquid nitrogen and were stored at −70°C until use. Cryostat sections (5 μm) were dried at room temperature overnight and fixed in acetone at 0°C for 5 min. The immunofluorescence staining was performed using mouse polyclonal antibody against NV-F followed by fluorescein isothiocyanate–conjugated rabbit anti-mouse antibody (Jackson Immuno Research Laboratories). Double staining was performed by simultaneously staining the nuclei with DAPI (200 ng/mL). Confocal microscopy was performed using a Leica TCS SP2 Laser Scanning Spectral Confocal System.

RESULTS

Strategy to identify foreign sequences in the serum sample of a patient with non–A–E hepatitis. A 66-year-old man (patient L) received a diagnosis of colon cancer (adenocarcinoma in transverse colon) in December 1999 at Chang Gung Medical Center. He received a colectomy, which was later complicated by anastomosis leakage, sepsis, and gastric ulcer bleeding. After intensive medical treatment, including blood transfusion, the patient's condition was gradually stabilized. Unfortunately, an episode of acute hepatitis (peak ALT level, 284 U/L) with deep jaundice (bilirubin level, 19 mg/dL) occurred in July 2000. The patient was found to be negative for HBsAg, IgM anti-HAV antibody, IgM anti-HBc antibody, anti-HDV antibody, and anti-HCV antibody. The patient also tested negative for HEV RNA and HCV RNA. The serum sample obtained at this point was used for molecular cloning of foreign sequences.

To identify foreign sequences in the serum sample, total serum DNA or RNA was extracted. The nucleic acid was then amplified (by PCR or RT-PCR) using random primers. The amplified product was subsequently subjected to a second-step PCR using designed primers (see Patients, Materials, and Methods). To eliminate sequence derived from human chromosomes, the resulting clones were hybridized with the probes generated from cytoplasmic RNA of normal liver tissue. All positively hybridized clones were discarded. The remaining 195 clones were sequenced using an automatic DNA sequencer. The sequencing data were compared with the human genome sequence, as well as with sequences in GenBank, by use of NCBI BLAST. Only 3 clones were found to be of nonhuman origin. One of the sequences, derived from the DNA extract, contained an open reading frame with incomplete 5′ and 3′ ends and was temporarily named NV-F (figure 1*A*). The sequence potentially encoded a peptide with incomplete amino- and carboxy-termini. Four primers, NV-F1 to NV-F4, were designed for the nested PCR assay. By use of this assay, this sequence was found to be absent in the chromosomal DNA extracted from HepG2 cells, Daudi cells, and 3 different sources of human peripheral blood mononuclear cells.

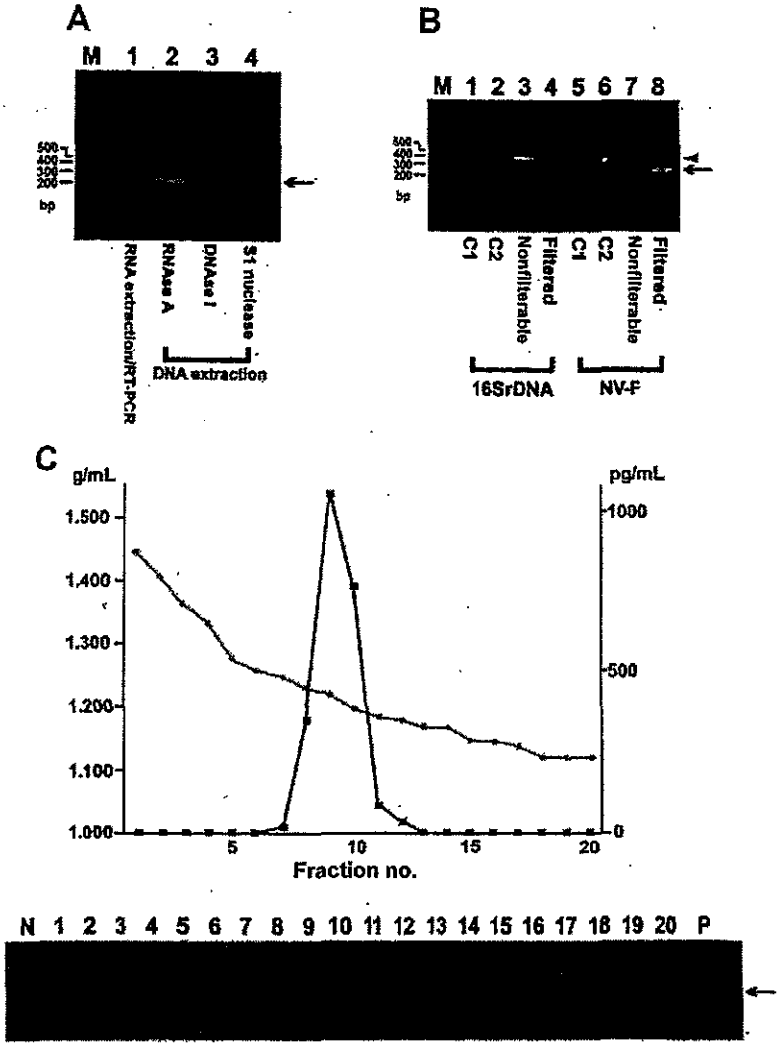

Figure 4. Characterization of the NV-F agent. *A,* Extraction of nucleic acid from the serum sample by either the RNA extraction *(lane 1)* or the DNA extraction method *(lanes 2–4).* After RNA extraction, reverse transcription (RT) polymerase chain reaction (PCR) for detection of the NV-F sequence was performed, without any intermediate step *(lane 1).* After DNA extraction, the extracted sample was treated with RNAse A *(lane 2),* DNAse I *(lane 3),* or S1 nuclease *(lane 4)* before subsequent PCR assay for NV-F sequence. M, molecular weight marker. The arrow indicates the PCR product of NV-F. *B,* Size assessment of the NV-F agent. Serum containing the NV-F agent was mixed with *Escherichia coli* and passed through a filter with a pore size of 0.2 μm. PCR was performed to detect 16S ribosomal DNA of *E. coli (lanes 1–4)* or NV-F *(lanes 5–8)* in filtered (*lanes 2, 4, 6,* and *8*) or nonfilterable (*lanes 1, 3, 5,* and *7*) fractions. An aliquot of serum negative for the NV-F sequence (C1 and C2) was assayed in parallel as a mock control. The arrowhead indicates the PCR product of 16S ribosomal DNA, and the arrow indicates the PCR product of NV-F. *C,* Cesium chloride gradient analysis for the NV-F agent. A serum sample positive for both hepatitis B virus (HBV) DNA and the NV-F sequence was used for cesium chloride gradient analysis. Twenty fractions were collected. All were sent for both HBV DNA quantitation *(upper panel)* and 1-step PCR (for the NV-F sequence) followed by Southern blot analysis *(lower panel).* Circles denote densities, and squares denote HBV DNA levels. The arrow indicates the PCR product of the NV-F sequence. N, negative hybridization control (1 ng of pCR2.1-TOPO); P, positive hybridization control (1 ng of the NV-F sequence).

Detection of the NV-F sequence in patients with non–A–E hepatitis. Serum samples from 4 groups of patients were included for the detection of the NV-F sequence (figure 1*B*). The sequence was detected in 5 (2.8%) of 180 healthy individuals. In contrast, NV-F was present in 17 (24.6%) of 69, 21 (14.0%) of 150, and 42 (28%) of 150 patients with non–A–E hepatitis, chronic hepatitis B, and chronic hepatitis C, respectively. One of the 17 patients whose serum was positive for NV-F had fulminant hepatitis. This was a 47-year-old male (patient F) who had non–A–E hepatitis accompanied by intermittent high fever and chills in May 2003. He was admitted for liver biopsy and further clinical investigation. Liver decompensation with

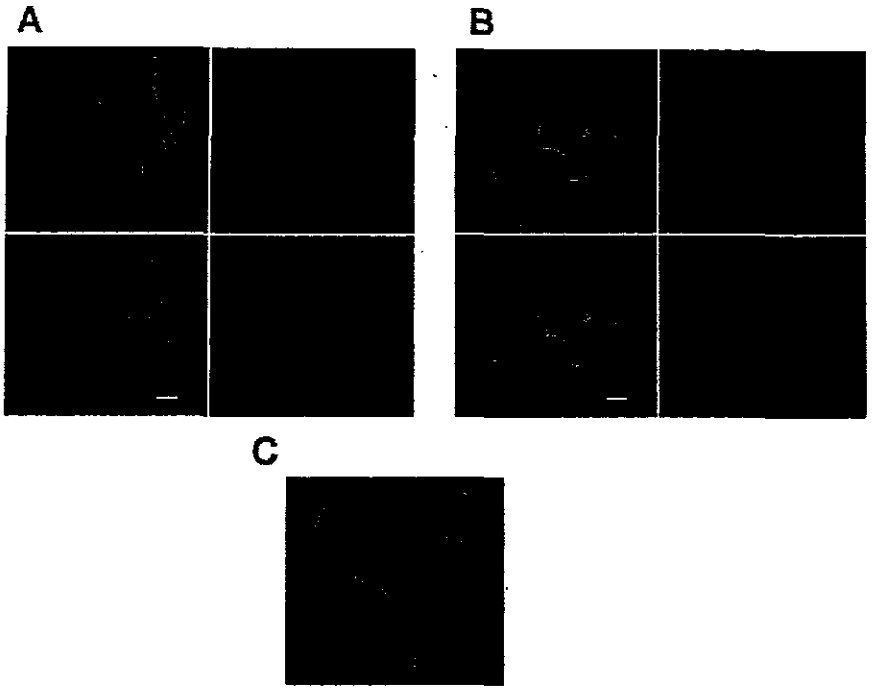

Figure 5. Detection of NV-F antigen in the liver biopsy sample from a patient with fulminant non–A–E hepatitis (patient F). Two different sections (*A* and *B*) from the same biopsy are shown. Positive cells in panel B are shown at higher magnification in panel C. Immunofluorescence analysis was performed using anti–NV-F antibody *(left upper panel)* and DAPI *(right upper panel)* for double staining. The pictures were overlapped using confocal microscopy *(left lower panel)*. A negative control *(right lower panel)* using preimmune serum for staining was included. Scale bar, 20 μm.

massive ascites, bilateral pleural effusion, and consciousness disturbance developed 10 days after onset. Thereafter, the patient's condition improved progressively without the need for any specific treatment, and he finally recovered completely. No known infectious agent was found throughout the course of the illness. Serial serum samples were obtained from this patient; his serum was found to be positive for the NV-F sequence during the early stage of the hepatitis flare, but it became negative thereafter (figure 2).

Expression of NV-F peptide and detection of anti–NV-F antibody. The putative coding sequence flanked by NV-F1 and NV-F4 was used to express a fusion protein containing the putative NV-F antigen, polyhistidine, and an Xpress epitope, using insect cells. After purification, a doublet was found in the protein gel, which could also be seen by Western blot using anti-Xpress antibody (figure 3*A*). A mouse polyclonal antibody was then raised against the fusion protein. This antibody recognized a single protein species when only the NV-F peptide (no other fusion parts) was expressed in the insect cells (figure 3*B*, *lane 1*). By use of this peptide as an antigen, anti–NV-F antibody in serum from patient L was assayed. Western blot analysis revealed only 1 protein species (figure 3*B*, *lane 3*). The doublet derived from the fusion protein was, therefore, likely a result of partial degradation. Serum samples were subsequently examined for the presence of anti–NV-F antibody, using the insect cell lysate containing NV-F peptide (no other fusion parts) as well as the purified NV-F fusion protein as an antigen. The results obtained by use of the 2 methods were consistent. It was found that anti–NV-F antibody was present in 49 (75.4%) of the 65 patients whose serum was found to be positive for the NV-F sequence, including patient L and patient F. Of the 49 positive samples, 15 were from patients with non–A–E hepatitis, 16 were from those with chronic hepatitis B, and 18 were from those with chronic hepatitis C. In contrast, anti–NV-F antibody was undetectable in 90 patients whose serum was negative for the NV-F sequence (49 healthy individuals, 10 patients with non–A–E hepatitis, 11 patients with chronic hepatitis B, and 20 patients with chronic hepatitis C).

Characterization of the NV-F–associated agent. The nucleic acid was extracted from the serum sample from patient L, using either a DNA or an RNA extraction method. The nucleic acid was then digested by DNAse I, RNAse A, or S1 nuclease before the PCR assay. The results showed that the NV-F sequence was present only in the nucleic acid fraction that was extracted using the DNA extraction method. The NV-F sequence was resistant to RNAse A digestion but was sensitive to DNAse I and S1 nuclease digestion (figure 4*A*). To estimate the size of the NV-F–associated agent, the serum sample was mixed with 10^5 *E. coli*

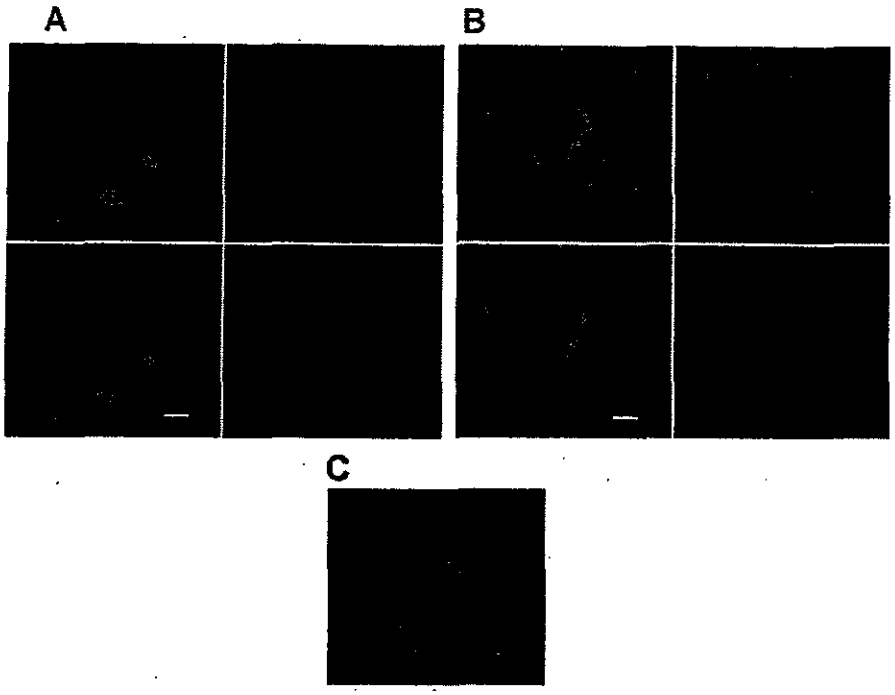

Figure 6. Detection of NV-F antigen in the liver biopsy sample from a patient with NV-F and hepatitis B virus coinfection (patient B). See the legend to figure 5 for further details.

organisms and passed through a filter with a pore size of 0.2 μm. The nonfilterable material was resuspended in PBS and analyzed in parallel with the filtered portion. The result indicated that the putative particles containing the NV-F sequence were smaller than 0.2 μm (figure 4*B*).

It was found that, in some patients with chronic hepatitis B, coinfection with the NV-F agent and HBV occurred. A 36-year-old male (patient B from the chronic hepatitis B group) who had chronic hepatitis B with mild activity for >2 years came to our clinic to undergo a liver biopsy for fibrosis staging. A PCR assay revealed that his serum was also positive for NV-F. The serum sample from patient B was subjected to cesium chloride gradient analysis. The gradients were fractionated and assayed for the presence of HBV DNA (using a quantitative test) and the NV-F sequence (using 1-round PCR followed by Southern blot analysis). Two peaks of NV-F sequence were present, one in the fractions of 1.33–1.39 g/mL and the other in the fractions of 1.22–1.25 g/mL (figure 4*C*). The peak HBV DNA concentration was found in the fraction of 1.19–1.21 g/mL, indicating that the HBV particles were slightly lighter than the NV-F–associated particles. This experiment was repeated using serum samples from 3 other patients with NV-F–associated hepatitis, and the results were consistent.

Immunofluorescence analysis. By use of the NV-F fusion protein expressed in insect cells, mouse anti–NV-F antibody was developed for immunofluorescence analysis. This antibody specifically detected the putative NV-F antigen (figure 3*B*). Immunofluorescence analysis was performed on the liver biopsy tissue obtained from patient F (figure 5) and patient B (figure 6). It was found that the antigen was distributed either in a speckle pattern or homogeneously in the cytoplasm of hepatocytes. Furthermore, positive staining was also observed in the perinuclear area (or on the nuclear membrane) in most positively stained cells.

DISCUSSION

Owing to technological advances in molecular biology, 5 major hepatitis viruses (HAV to HEV) have been discovered. The etiology of chronic hepatitis can thus be determined in a great majority of patients. Despite this achievement, the cause of chronic hepatitis remains elusive in ~5% of patients [5, 14]. Furthermore, in acute hepatitis, the proportion of patients with undetermined etiology is even higher [3, 15]. In Taiwan, the HBV carrier rate is ~15%, and more than half of the inpatients in Taiwan with acute hepatitis are seropositive for HBsAg [3]. It is believed that acute exacerbation of hepatitis B in chronic HBV carriers is responsible for the majority of acute hepatitis flares [16]. Even though the proportion of patients with non-B hepatitis is small, the etiology of acute hepatitis remains undetermined in 15.9% of our inpatients, suggesting the existence of other, unidentified hepatitis viruses [3]. In this study, we have

identified a fragment of DNA sequence (NV-F) in the serum of a patient with non–A–E hepatitis. Only 2.8% of healthy individuals carried this sequence in their serum, whereas 24.6% of patients with non–A–E hepatitis were positive for NV-F. In this study, we did not exclude patients with nonalcoholic steatohepatitis from the non–A–E hepatitis group, nor did we exclude patients with fatty liver [17, 18]. It is possible that the prevalence of NV-F would be even higher if such patients were excluded. Interestingly, a high prevalence of NV-F is also observed in patients with chronic hepatitis B or C, indicating that coinfection with NV-F and either HBV or HCV frequently occurs. Similarly, when HCV was initially discovered, many studies on the seroprevalence of HCV indicated that HCV was found in >10% of HBV-infected patients worldwide [19]. The prevalence might be underestimated, since HCV superinfection exerts a suppressive effect on HBV and enhanced seroclearance of HBV [20]. Coinfection with HBV or HCV was also commonly found in patients with GBV-C, TTV, and SEN virus infection. Supposedly, such a high percentage of coinfection is attributed to a common transmission route. The effect of NV-F superinfection on chronic hepatitis B or C is not clear at this time. A detailed clinical analysis is needed to answer this question. Despite a high prevalence of the NV-F agent in non–A–E hepatitis, it is still questionable whether NV-F is the direct cause of hepatitis. Since NV-F frequently coinfects with HBV or HCV, it remains possible that NV-F coinfects with a yet-unidentified virus in patients with non–A–E hepatitis and that it is the unidentified virus that serves as the direct cause of hepatitis. In this study, we have provided 2 pieces of evidence suggesting that NV-F might contribute, at least in part, to the hepatitis activity. First, in patient F, NV-F viremia occurred concurrently with the hepatitis flare, and the NV-F agent was cleared from the serum after recovery from the disease. This temporal relationship argues for a causative role of NV-F in non–A–E hepatitis. Second, the NV-F antigen was found in the cytoplasm of hepatocytes, suggesting that this agent is hepatotropic. The presence of a foreign antigen in the liver cells frequently results in an inflammatory reaction—namely, hepatitis—unless other unknown mechanisms are involved to deter the host immune response. Further immunological study is needed to understand the mechanism of NV-F–associated non–A–E hepatitis.

At this time, the biological nature of the NV-F agent has not been completely defined. Our data indicate that it is smaller than 0.2 μm, forms 2 buoyant densities in a cesium chloride gradient, and possesses single-stranded DNA. These features suggest that the NV-F agent is possibly a virus. The presence of 2 densities in cesium chloride gradient analysis is sometimes observed in an enveloped virus. A possible explanation is that some particles containing only the nucleocapsid (but not the envelope) form the band with the higher density. However, owing to an extremely low serum concentration of NV-F, the attempt to visualize the particles by electron microscopy failed. Southern and Western blot analysis using the remaining liver biopsy samples submitted for this study from patients L and B (only 3 mm in length) failed to demonstrate the viral genome and protein. A larger piece of tissue, such as surgically removed liver tissue, may be required to achieve this goal. A BLAST search showed that none of the known sequences shared sequence homology with NV-F. Further extension of the 5′ and 3′ ends of the NV-F sequence is, thus, progressing very slowly. The best-known single-stranded DNA viruses are parvoviruses and circoviruses. It is possible that NV-F belongs to a class of virus distantly related to one of these 2 families. Alternatively, it may represent a new class of agents that has no known close relatives.

In summary, we have discovered a novel single-stranded DNA sequence that is associated with human hepatitis, including in a patient with fulminant non–A–E hepatitis. The NV-F agent is hepatotropic and likely belongs to a novel class of viruses. Finally, this virus frequently coinfects with HBV or HCV in patients with chronic hepatitis.

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医薬品 研究報告 調査報告書

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研究報告の概要

○オランダにおける血小板輸血の臨床的安全性に関する手指消毒法、初流血除去バッグ、細菌スクリーニングの効果
背景:血液製剤の細菌汚染は、致死的な輸血副作用が発現する大きな危険性がある。好気的および嫌気的培養(BacT/ALERT, bioMerieux)による濃厚血小板(PC)の細菌スクリーニングは、2001年10月にオランダに導入された。
実験デザインおよび方法:2002年11月、70%イソプロピルアルコールのダブルスワブで消毒するという全国統一の皮膚消毒法が導入された。ある施設では、日常的に初流血除去バッグを使用して初流血20～30mLを除去していた。
結果:2002年から2003年にかけて、プールバフィーコート由来のPC合計113,093のスクリーニング検査が行われた。新しい消毒法の導入後、0.85%は初回陽性であった。初回陽性が0.95%であったこれまでの消毒法と比較して、減少幅は小さかった。初流血除去バッグを使用していた施設では、細菌汚染の頻度が有意に低く、70%イソプロピルアルコールによる消毒法の導入前は0.50%、導入後は0.37%であった。また、アフェレーシスPC8000件のスクリーニング検査も行われ、初回陽性は24検体(0.30%)であった。
結論:初流血除去バッグの使用および、減少幅は小さいものの70%イソプロピルアルコールのダブルスワブの使用によって、細菌汚染が減少した。予測された通り、大半を占める皮膚常在菌の汚染が減少した。初流血除去バッグと新たな消毒法を併用した場合、初回陽性の発現頻度は、供血者5名のプールPCと単一供血者由来のアフェレーシスPCと同程度のものであった。さらに、細菌検出システムとそれに伴う製剤回収手順は、特に急速に増殖する細菌によって汚染されたPCや赤血球の輸血による感染防止に効果的であることが示された。

報告企業の意見	今後の対応
初流血除去バッグの使用および70%イソプロピルアルコールのダブルスワブの使用によって、輸血用血液製剤の細菌汚染が減少したとの報告である。	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)における「本ガイドライン対象以外の病原体の取扱い イ. 細菌」に準じ細菌感染が疑われる場合の対応を医療機関に周知している。 今後も情報の収集に努める。採血時の初流血除去、白血球除去の導入とともに細菌を不活化する方策についても検討を進める。

使用上の注意記載状況・その他参考事項等

解凍赤血球濃厚液「日赤」
照射解凍赤血球濃厚液「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

Effects of skin disinfection method, deviation bag, and bacterial screening on clinical safety of platelet transfusions in the Netherlands

Dirk de Korte, Joyce Curvers, Wim L.A.M. de Kort, Tiny Hoekstra, Cees L. van der Poel, Erik A.M. Beckers, and Jan H. Marcelis

BACKGROUND: Bacterial contamination of blood products is a great hazard for development of fatal transfusion reactions. Bacterial screening of platelet concentrates (PC) by aerobic and anaerobic culturing (BacT/ALERT, bioMérieux) was introduced in the Netherlands in October 2001.

STUDY DESIGN AND METHODS: In November 2002, a nationwide, uniform skin cleansing method was introduced with a double-swab disinfection with 70 percent isopropyl alcohol. One location routinely used an integrated diversion bag to collect the first 20 to 30 mL.

RESULTS: Over the calendar years 2002 and 2003, in total 113,093 PCs derived from pooled buffy coats were screened. After introduction of the new disinfection method, 0.85 percent were initially positive. This was a small reduction compared to the previous disinfection methods under which 0.95 percent were initially positive. The location with use of the diversion bag showed a significantly lower frequency of bacterial contamination, with 0.50 percent before and 0.37 percent after introduction of 70 percent isopropyl alcohol. In addition 8000 apheresis PCs were also screened, showing 24 initially positive samples (0.30%).

CONCLUSION: The use of the diversion bag and, to a lesser extent, the use of double swabs with 70 percent isopropyl alcohol, led to a reduction of contamination. As expected, predominant contamination with resident skin bacteria was reduced. The combination of diversion bag and new disinfection led to a frequency of initial positive results for pooled five-donor PCs, which is similar to that of single-donor apheresis PCs. Furthermore, the bacterial detection system and associated product recall procedures have been shown to be effective in preventing transfusion of contaminated PCs and/or related red cells, especially for rapidly growing bacteria.

Since the dramatic reduction of transfusion-transmitted viral infections though screening for various blood-borne viruses, transfusion-related bacterial infections have become one of the major risks of transfusion. Bacterial contamination is considered to be, after clerical errors, the second most common cause of death from transfusion, with mortality rates for platelet (PLT)-related sepsis ranging from 1 in 20,000 to 1 in 100,000 donor exposures.[1-3] In contrast, the current frequencies of virus transmission via blood components are estimated as 1 in 1,800,000 for hepatitis C virus, 1 in 220,000 for hepatitis B virus, and 1 in 2,300,000 for human immunodeficiency virus.[4,5] Therefore, additional screening of blood products for bacterial contamination is under consideration or has already been implemented in many countries, both in Europe and in North America.[6]

Because of their storage at room temperature, PLT products are most sensitive for bacterial contamination and thus a logical choice to start screening. In the Neth-

ABBREVIATIONS: CNS = coagulase-negative staphylococci; IPA = isopropyl alcohol; PC(s) = platelet concentrate(s); T5 = PCs prepared from pools of five buffy coats; TTBI = transfusion-transmitted bacterial infection.

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erlands, nationwide screening for bacterial contamination in 100 percent of PLT products was introduced at the end of 2001. All Dutch blood banks use the BacT/ALERT culturing system (bioMérieux [formerly Organon Teknika], Boxtel, the Netherlands), with a standardized protocol.

It has previously been shown that in the majority of positive cultures, bacterial contamination is a result of resident skin flora[7] most likely originating from the venipuncture plug.[8,9] Although hygienic precautions are taken to prevent contamination during collection, a further reduction of the number of products with bacterial contamination is desirable. To reduce the risk of contamination by skin flora two strategies can be used: 1) diversion of the first aliquot of the donation and 2) improvement of skin disinfection.

Regarding the first method we, as well as others, have previously shown that diversion of the first 10 mL of a whole-blood donation reduces the incidence of bacteria in the remaining whole-blood unit.[8,10,11] The effect of this diversion, however, on bacterial contamination of the final product, PLT concentrates (PCs) prepared from pooled buffy coats, has not yet been reported.

Considering skin disinfection, iodine is the most effective disinfectant. Because it is considered to be a donor-unfriendly agent, however, isopropyl alcohol (IPA) is the next best choice. McDonald and coworkers[12] have shown that improved skin disinfection methods drastically reduced the number of remaining bacteria on the phlebotomy puncture site—especially those methods with a double-swab method, with the best results for the combination of IPA and iodine. For this approach too, the final effect on bacterial contamination of buffy coat–derived PCs has not yet been reported.

From January 2002, at collection centers of the Sanquin Blood Bank Region Southeast (Nijmegen, the Netherlands), a collection system with an integrated diversion bag was used to divert the first 20 to 30 mL of the whole-blood donation. This volume was subsequently used for infection disease and immunohematology testing. All other collection centers of the Sanquin Blood Banks used standard whole-blood collection systems without a diversion bag. In October 2002, a standardized skin disinfection method, with 70 percent IPA in a double-swab method, was introduced in all the collection centers of the Sanquin Blood Banks (including Nijmegen). During the whole period all apheresis PCs were collected with a diversion pouch included in the system.

In this report we evaluate data on bacterial contamination in the Netherlands for all apheresis PCs and PCs prepared from pools of five buffy coats (T5), collected in 2002 and 2003. The large numbers enable us to make a reliable judgment of the effect of diversion and/or changed disinfection method on the final degree of bacterial contamination of PC.

MATERIALS AND METHODS

Blood collection with or without diversion

Whole blood was collected under standard blood banking conditions with either a four-bag top and bottom citrate phosphate dextrose (CPD)-saline adenine glucose mannitol (SAGM) red cell (RBC) inline filter system (Compoflex, Fresenius Hemocare, NPBI International, Emmer-Compascuum, the Netherlands) or a comparable system (Baxter PL146-CPD-70 mL 3-Optipure, Baxter, Utrecht, the Netherlands).

Sanquin Blood Bank Region Southeast (Nijmegen, the Netherlands) used a five-bag top and bottom CPD-SAGM RBC inline filter system (Compoflex, Fresenius Hemocare, NPBI International) including an integrated sample bag (T3941, Fresenius Hemocare), in which diversion of the first 20 to 30 mL of the donation was performed. After collection of the first volume, a clamp was set and the sample bag was sealed. Donation proceeded normally in the collection bag with CPD. All other collection centers collected blood for infectious diseases and immunohematology testing after donation was completed via a sampling site attached to the collection system.

Processing to PCs

Whole blood is processed similarly at all collection centers after collection, with rapid cooling to 20°C and overnight storage at this temperature.[13] Briefly, after a hard centrifugation of the whole blood, the buffy coats are collected. Five buffy coats (same blood group) are pooled and mixed with either 300 mL of plasma or additive solution (AS; PAS I, Baxter). The pooled buffy coats are centrifuged again (soft centrifugation) to produce T5 products. Preparation procedures for T5 varied slightly between regions, but these differences (type of leukodepletion filter and storage container) are not likely to cause variations in the degree of bacterial contamination.

The blood bank locations of Rotterdam (Region Southwest), Utrecht (Region Northwest), and Nijmegen (Region Southeast) used PAS II as AS in the T5 products; all other blood bank locations used plasma for pooling.

Skin disinfection

Before October 2002 skin disinfection was performed with various methods in the Sanquin collection centers, referred to as the "old skin disinfection method." Most centers (>95% of total collections) used a single swab method with 70 percent alcohol–0.5 percent chlorhexidine or 70 percent IPA, but some small centers used single swabs with iodine tincture, whereas one small center used a double-swab method with 70 percent alcohol–0.5 percent chlorhexidine. Because no differences were found between the various centers for the old methods,

the data for the old methods have been pooled (with and without exclusion of the center with the diversion bag). Starting from October 2002, a uniform disinfection method was introduced nationwide with a 30-second spaced double-swab method with 70 percent IPA. Sanquin Blood Bank Region Southwest introduced this new disinfection method in October 2002, the Regions Northeast and Southeast did so in November 2002, and Region Northwest introduced it in January 2003.

Screening on bacterial contamination

For the screening on bacterial contamination, within 2 hours after preparation of a T5 (this is 16-22 hr after collection) or within 12 hours after collection of the apheresis product, both aerobic and anaerobic culture bottles were inoculated (approx. 7.5 mL per bottle; range, 5-10 mL). Inoculation was performed under aseptic conditions with a laminar airflow cabinet, as reported previously.[7]

Culture bottles were incubated at 35°C in the BacT/ALERT system until flagged positive or up to 7 days if negative (also in those cases were the corresponding bottle became positive). Positively flagged bottles were sent to regional reference laboratories for determination by plating the sample. PCs and RBCs related to an initially positive bottle were taken out of inventory or recalled. Corresponding RBCs, if available from stock or recall, were cultured in the BacT/ALERT (for 7 days; both aerobic and anaerobic bottle), and positively flagged bottles were also sent to the reference laboratories for determination.

Study design and statistical analysis

Both initial culture test results and data on the microbiologic determination have been included in analyses of the results over the complete calendar years 2002 and 2003. The results were used to test the effects of the new disinfection method and/or the diversion on the degree of contamination. The results of the center with the diversion bag were compared to the pooled results of all other centers not with the diversion bag.

Data showing a positive initial test, but without determination results, have been labeled as missing. If the determination test could not detect microorganisms, the initial test result has been labeled as negative determination culture.

Because the hypothesis was that the contamination degree would be decreased after introduction of diversion and/or new disinfection, differences in frequency of bacterial contamination between the (sub)groups were tested with a one-sided chi-square test unless indicated otherwise. Logistic regression was used to calculate the odds ratios (OR) for risk of bacterial contamination with the different methods (diversion and disinfection). Multivariate logistic regression was performed to correct for possible confounding effects, such as seasonal effects and AS.

All statistical analyses were performed with computer software (SPSS, version 11.0, SPSS Inc., Chicago, IL).

RESULTS

Number of contaminated products

In 2002 and 2003, a total of 122,907 PCs were tested in the BacT/ALERT system, with an aerobic and an anaerobic bottle per product, each inoculated with 7.5 mL of PC. The majority of these PCs were T5, but 1814 products were made by pooling three buffy coats, with an initially positive signal rate of 0.39 percent (n = 7). A total of 8000 apheresis PCs were tested, with 0.30 percent (n = 24) initially positive in the BacT/ALERT system. Because of their small numbers, the products made by pooling three buffy coats are left out of further analysis, and the apheresis products are presented as separate group, with limited data analyses, leaving 113,093 T5 products.

In approximately 10 percent of initially positive results, with some variation per region, no microorganism could be cultured from the positive bottle. Infrequently, however, a microorganism could be isolated from one of the associated RBCs (9 of 106). For a limited number of initially positive samples no information was reported regarding determination culture (these are labeled as missing), but for the large majority (98%) data on determination were available.

Effects of diversion and disinfection methods

In Table 1 the frequency of bacterial contamination for the different collection methods is shown. Diversion was associated with a significant reduction of bacterial contamination from 0.95 to 0.50 percent (chi-square test, $p = 0.002$) with the old skin disinfection method and from 0.85 to 0.37 percent (chi-square test, $p = 0.001$) when the new disinfection method was applied. For collections without diversion, the new skin disinfection method compared to the old methods resulted in a mild reduction in initially positive samples from 0.95 to 0.85 percent (chi-square test, $p = 0.049$). For collections with diversion, the new skin disinfection method compared to the old method also resulted in a reduction in initially positive samples from 0.50 to 0.37 percent (chi-square test, $p = 0.18$).

Calculated on initially positive samples, diversion resulted in an OR of 0.47 (95% CI, 0.35-0.63; $p < 0.001$) with univariate logistic regression. Multivariate logistic regression, with correction for disinfection method, time of screening (season), and AS resulted in an OR of 0.49 (95% CI, 0.36-0.67; $p = 0.003$).

For the new disinfection, the OR was 0.88 (95% CI, 0.77-1.00; $p = 0.05$) in univariate analysis and 0.87 (95% CI,

TABLE 1. Degree of contamination in subsets of T5 products*

Collection method	No diversion	Diversion	Total
Old skin disinfections			
Total tested	42,582	4,362	46,944
Initially positive (%)	405 (0.95)	22 (0.50)	427 (0.91)
Positive determination culture (%)	373 (0.88)	18 (0.41)	391 (0.84)
Negative determination culture (%)	26 (0.06)	4 (0.09)	30 (0.06)
Missing subculture data	6	0	6
70 percent IPA skin disinfection			
Total tested	59,400	6,749	66,149
Initially positive (%)	505 (0.85)	25 (0.37)	530 (0.80)
Positive determination culture (%)	427 (0.72)	17 (0.25)	444 (0.67)
Negative determination culture (%)	68 (0.11)	8 (0.12)	76 (0.11)
Missing subculture data	10	0	10
Total test period			
Total tested	101,982	11,111	113,093
Initially positive (%)	910 (0.89)	47 (0.43)	957 (0.85)
Positive determination culture (%)	800 (0.78)	35 (0.32)	835 (0.74)
Negative determination culture (%)	94 (0.09)	12 (0.11)	106 (0.09)
Missing subculture data	16	0	16

* Absolute numbers, within parentheses: percentages of total tested.

0.76-0.99; p = 0.03) with multivariate analysis corrected for time of screening (season), AS, and diversion. Because of the study design, correction for the region was not possible, but no significant differences between the regions were found. Removing individual centers from the analysis did not alter any of the conclusions and only marginally affected estimated effect sizes and CIs.

The effects of the various interventions have been evaluated, either with the percentages of initially positive samples or with the percentage of positive samples resulting in a positive determination culture. The percentage of initially positive samples determines the effect of the interventions on the numbers of PCs and related RBCs blocked by the screening and thus the consequences for blood banking logistics. The positive determination cultures reflect the effect of the interventions on the final degree of bacterially contaminated PCs. The ORs were slightly lower (with lower p values) when calculated on the number of positive determination cultures, owing to the increase in negative determination cultures after introduction of diversion and/or the new disinfection method.

Plasma compared to AS

At three collection centers (Rotterdam in Region Southwest, Utrecht in Region Northwest, and Nijmegen in Region Southeast), T5 products were prepared in a mixture of PLT AS (PAS II, Baxter) and plasma (65:35 ratio, vol/vol; n = 35 812). We have compared the contamination in PAS II products without diversion with plasma products without diversion to evaluate the effect of AS on the degree of contamination.

The frequency of bacterial contamination was slightly reduced in PAS II PLT products (0.86%) compared to plasma products (0.91%; chi-square test, p = 0.44, not significant). This difference did not change after the introduction of the 70 percent IPA.

Determination results

Table 2 summarizes the different species found in the contaminated T5 products, with classification in some origin-related groups. The majority of detected microorganisms were skin bacteria, mainly diphtheroids or coagulase-negative staphylococci (CNS; and other Gram-positive cocci). The other bacteria belong either to the transient skin bacteria or to the gastrointestinal tract.

After diversion, contamination with skin flora (Table 2; both diphtheroids [chi-square test, $p < 0.001$] and the CNS plus group [chi-square test, p = 0.001]) was significantly reduced, whereas the other groups were not significantly reduced. The fact that no bacteria belonging to the gastrointestinal tract were found after diversion is probably due to their low frequency in combination with the relatively small numbers tested. The fraction of initially positive bottles with a negative determination culture was higher with diversion, although not significantly so (chi-square test, p = 0.36).

When comparing the old disinfection method with the new disinfection method (without diversion), a significant reduction of the CNS plus group was found (Table 2; chi-square test, $p < 0.001$), but no reduction of diphtheroids. Contamination with other bacteria was slightly reduced; however, this difference was significant only for *Staphylococcus aureus* (chi-square test, p = 0.02). As for diversion, an increase in the fraction with a negative determination culture was observed (chi-square test, p = 0.002).

Some significant differences in the type of microorganisms were detected between plasma and PAS II PLT

TABLE 2. Determination of initially positive samples*

Variable	Old disinfection, no diversion	New disinfection, no diversion	Diversion (both disinfections)	Totals
Number of PCs tested	42,582	59,400	11,111	113,093
Initially positive bottles	405	505	47	957
Missing determination	6	10	0	16
Skin flora				
Diphtheroids†	160 (0.38)	230 (0.39)	18 (0.16)	408
CNS plus‡	153 (0.36)	119 (0.20)	12 (0.11)	284
S. aureus§	9 (0.02)	3 (0.005)	1 (0.01)	13
Transient skin flora				
Bacillus spp.‖	25 (0.06)	35 (0.06)	4 (0.04)	64
Gastrointestinal tract flora				
Gram-negative rods¶	8 (0.02)	9 (0.02)	0	17
Streptococci	5 (0.01)	1 (0.002)	0	6
Peptostreptococci	9 (0.02)	15 (0.03)	0	24
Residual group				
Others	4 (0.01)	15 (0.03)	0	19
No microorganism	26 (0.06)	68 (0.11)	12 (0.11)	106

* Values given as absolute numbers (percentage of total tested samples), with an additional column adding up absolute numbers.
† Diphtheroids include: *Propionibacterium* spp. (mainly acnes), *Corynebacterium* spp., and unspecified diphtheroid rods or Gram-positive rods (not spore forming).
‡ CNS plus include all coagulase-negative *Staphylococcus* subspecies (mainly epidermidis) and Gram-positive cocci (less than 10% of the total; aerococci [2]; micrococci [5], and unspecified [16]).
§ Although there are limited reports on *S. aureus* contamination originating from endogenous bacteremia in the donor.
‖ *Bacillus* spp. (roughly 50% *B. cereus*), including some unspecified spore formers (12).
¶ Various species, i.e., pseudomonas (1), brevundimonas (1), flavomonas (2), bacterioides spp. (4), salmonella (1), proteus vulgaris (1), and some unspecified (6 aerobic, 1 anaerobic).

products. Both *S. aureus* and Gram-negative rods had a significantly lower frequency in PAS II products (chi-square test, $p = 0.01$, two-sided). Moreover, the frequency of initially positive bottles with a negative determination culture was lower in PAS II (chi-square test, $p = 0.01$, two-sided). These differences might be explained by a lower initial load with bacteria, because less potentially contaminated whole blood–derived material is used.

Aerobic and anaerobic bottles

Table 3 shows the mean time until the aerobic and anaerobic bottles became positive, in relation to the determination result. In general, aerobic bottles became positive sooner than anaerobic bottles. From the positive aerobic bottles only 35 (11%) turned positive after 5 days, whereas from the positive anaerobic bottles 221 (35%) turned positive after 5 days. Except for diphtheroid (more particularly the *Propionibacterium* spp.) and peptostreptococci species, most bacteria did not show a preference for either the aerobic or the anaerobic bottles (Table 3). This is in agreement with the results of spiking studies by Brecher and colleagues[14] showing for most bacteria growth in both the aerobic and the anaerobic bottle. During the test period, only 46 positively flagged units were reported to become positive in both the aerobic and the anaerobic bottle. It must be kept in mind that every negative bottle (also in case the corresponding bottle became positive) was cultured for 7 days.

Apheresis products

Table 4 shows some details for the 8000 apheresis PCs, all collected with an apheresis set including a diversion pouch. Owing to small numbers, the 95 percent CIs overlap, and no significant differences were found between the old and new disinfection methods. As for the T5, the percentage of positively flagged bottles without microorganism in the determination culture was higher after introduction of the new disinfection method (chi-square test, $p = 0.26$). In the apheresis PCs in two cases a Gram-negative rod was detected (0.03%; an *Escherichia coli* and a *Bacteroides* spp.), in one case a *Bacillus* spp. (0.01%), and in two cases a *Streptococcus* (0.03%). In eight cases the bacteria belonged to the CNS plus group (0.1%), in three cases the bacteria belonged to the diphtheroids group (0.04%), and in seven cases a negative determination culture was obtained (0.09%). These frequencies were comparable to those found in the T5 products after diversion (Table 2), except for diphtheroids (0.04% vs. 0.16% in T5).

Follow-up procedures after positive flagging

PCs. For 790 (83% of total) positively flagged T5, data on recall procedures were available (Table 5). From these units, 386 (49%) units had to be recalled and 404 units were still at the blood bank. The majority (88%) of the recalled products had already been transfused and only 45 of the recalled products could be prevented from being transfused (Table 5). Positively flagged units, which were

TABLE 3. Growth characteristics for different bacteria species

Variable	Total number of bottles	No data on time until positive	Both bottles positive	Aerobic bottle		Anaerobic bottle	
				Time until positive*	Number	Time until positive*	Number
Total	957	38	46	1.8	330	4.0	635
Skin flora							
Diphtheroids†	408	5	4	3.7	45	5.0	362
CNS plus‡	284	2	26	1.1	165	2.6	143
S. aureus§	13	0	0	0.9	6	1.3	7
Transient skin flora							
Bacillus spp.‖	64	1	7	1.7	41	2.1	29
Gastrointestinal tract flora							
Gram-negative rods¶	17	0	0	1.9	10	2.9	7
Streptococci	6	0	3	2.8	4	3.0	5
Peptostreptococci	24	1	0		0	3.9	23
Residual group							
Others	19		3	1.3	9	4.0	13
No microorganism	106	13	3	2.3	50	2.4	46
Missing determination	16	16					

* Mean time in days until positive signal with BacT/ALERT.
† Diphtheroids include: *Propionibacterium* spp. (mainly acnes), *Corynebacterium* spp., and unspecified diphtheroid rods or Gram-positive rods (not spore forming).
‡ CNS plus include all coagulase-negative *Staphylococcus* subspecies (mainly epidermidis) and Gram-positive cocci (less than 10% of the total; aerococci [2]; micrococci [5], and unspecified [16]).
§ Although there are limited reports on *S. aureus* contamination originating from endogenous bacteremia in the donor.
‖ *Bacillus* spp. (roughly 50% *B. cereus*), including some unspecified spore formers (12).
¶ Various species, i.e., pseudomonas (1), brevundimonas (1), flavomonas (2), bacterioides spp. (4), salmonella (1), proteus vulgaris (1), and some unspecified (6 aerobic, 1 anaerobic).

TABLE 4. Degree of contamination in subsets of apheresis products*

Variable	Old skin disinfections	70% IPA skin disinfection	Total test period
Total number tested	3037	4963	8000
Initially positive (%)	*7 (0.23)*	*17 (0.34)*	*24 (0.30)*
Positive determination culture (%)	6 (0.20)	12 (0.24)	18 (0.23)
Negative determination culture (%)	1 (0.03)	5 (0.10)	6 (0.08)
Missing subculture	0	1	1

* Absolute numbers, within parentheses: percentages of total tested. All apheresis products were collected with a set with diversion pouch.

already transfused at the moment of detection, had a mean time until detection of 4.7 days, whereas for the units still in the blood bank the mean time until detection was 2.2 days. Of 345 units with a positive signal in the first 48 hours of culture, only 27 units had already been transfused.

Table 5 also shows the recall information in relation to the various microorganisms. For the rapidly growing bacteria, most of the PCs were still in the blood bank inventory or could successfully be recalled. The majority of already transfused PCs were contaminated with diphtheroids, mainly flagging positive between Day 4 and Day 7 of culture.

During this surveillance period, no severe clinical effects of units flagged positive after transfusion were reported to Sanquin or the Dutch hemovigilance system (Transfusion Reactions in Patients; TRIP). Over the entire period, there were 165 follow-up reports (for every case a report was asked, but only in 40% a response was received) of transfused products corresponding to cultures flagged positive after release. In two cases mild clinical symptoms (fever) were reported, but no direct relation with the PLT transfusion was established. During the same period, two cases of sepsis were reported after transfusion of PCs with a negative signal in the screening[15] (also after 7 days of culturing). In both cases *Bacillus cereus* was identified as the causative microorganism, but it remains unclear whether this microorganism could have entered the PC during preparation or storage, due to pinholes in the bag, or whether it was definitely a false-negative result of the screening system (present in the sample, but not resulting in a positive flagging of the culture).

Related RBCs. In case of a positive signal in the bacterial screening of a pooled PLT product, corresponding RBCs were cultured. Data on RBC recall and cultures were available for 94 percent (901/957) of the initially positive flagged T5 units. In 40 percent of these cases there was a positive signal from the BacT/ALERT for one of the RBC units. In 85 percent of positive RBC units, the same microorganism as in the corresponding PC was found (majority diphtheroids). In the majority of cases where the microorganism was of different species, it belonged to the same group. Only in 27 cases was the microorganism found in

TABLE 5. Recall and transfusion details with respect to corresponding PCs and RBC units

Variable	Corresponding PC units				Related RBC units			
	Total number of PCs	Unknown*	Recall†	Transfused‡	Total number of RBC units	Unknown*	Recall†	Transfused‡
Total	957	167	386	341	4785	375	741	138
Skin flora								
Diphtheroids§	408	57	256	234	2040	76	553	113
CNS plusǁ	284	54	57	42	1420	85	92	12
S. aureus¶	13	0	0	0	65	2	0	0
Transient skin flora								
Bacillus spp.**	64	10	16	12	320	15	28	2
Gastrointestinal tract flora								
Gram-negative rods††	17	0	3	3	85	25	2	1
Streptococci	6	1	1	1	30	0	1	0
Peptostreptococci	24	3	14	12	120	2	16	3
Residual group								
Others	19	3	11	9	95	0	6	1
No microorganism	106	23	28	28	530	90	43	6
Missing determination	16	16			80	80		

* No data on recall available.
† Recall of products already released to hospitals.
‡ Already transfused at time of recall.
§ Diphtheroids include: *Propionibacterium* spp. (mainly acnes), *Corynebacterium* spp., and unspecified diphtheroid rods or Gram-positive rods (not spore forming).
ǁ CNS plus include all coagulase-negative *Staphylococcus* subspecies (mainly epidermidis) and Gram-positive cocci (less than 10% of the total; aerococci [2]; micrococci [5], and unspecified [16]).
¶ Although there are limited reports on *S. aureus* contamination originating from endogenous bacteremia in the donor.
** *Bacillus* spp. (roughly 50% *B. cereus*), including some unspecified spore formers (12).
†† Various species, i.e., pseudomonas (1), brevundimonas (1), flavomonas (2), bacteroides spp. (4), salmonella (1), proteus vulgaris (1), and some unspecified (6 aerobic, 1 anaerobic).

the RBCs totally unrelated to the organism as found in the PCs. This number included the 9 cases with no determination culture for the PC, whereas a microorganism could be isolated from one of the associated RBC units. The bacterial species in these cases were CNS plus (4), diphtheroids (4), and *Bacillus* spp. (1).

Of a total of 4505 RBC units (901 PCs each with 5 related RBC units), 741 RBC units had already been released and had to be recalled for culturing. The recall was successful in 68 percent of the cases. A total of 234 units were not available for culture, including 138 RBC units (relating to 99 PC units) that had already been transfused. For 69 of these 138 cases, one of the other related RBC units contained the same bacterium as found in the culture from the PC. From this it might be concluded that only in the remaining 69 cases possibly contaminated RBCs were transfused. These were mainly related to positive PLT cultures in which diphtheroids found (66/69). Because RBCs are stored at 4°C, however, it is not likely that these transfused RBCs contained harmful quantities of bacteria. This is supported by the fact that no transfusion reactions were reported for these units.

DISCUSSION

Based on the results of two calendar years (2002 and 2003) of bacterial screening of all PCs in the Netherlands, it can be concluded that interventions to reduce bacterial contamination were very effective, resulting in a reduction of initially positive cultures for pooled T5 products from 0.95 percent (no interventions) to 0.37 percent (two interventions: diversion and new skin disinfection). The 50 percent reduction in frequency upon diversion is very similar to that found in our previous whole-blood diversion study[10] and also corresponds with the reduction in contamination reported by McDonald and coworkers.[16] The double-swab 70 percent IPA disinfection method slightly enhanced the reducing effect on bacterial contamination of diversion, although this is of marginal significance and without synergy. The final frequency of 0.37 percent (95% CI, 0.24-0.55) positive cultures found for pooled T5 products after introduction of both diversion and new disinfection method is not significantly different from the 0.30 percent contamination rate for single donor apheresis PC (95% CI, 0.19-0.45), whereas the differentiation profile is also similar. This suggests that the relation between number of donor units and degree of bacterial contamination of the final PC, as described by Ness and colleagues,[1] no longer exists if special care is taken to avoid introduction of bacteria during collection. The finding that contamination is reduced to such a large extent by diversion has led to the introduction of the diversion pouch throughout the Netherlands, starting from July 2004.

Our current screening system for bacterial contamination of PC is highly sensitive by use of two bottles. The

fact that in less than 5 percent of positively flagged units both bottles were positive suggests that positive units have an initial bacterial contamination around the lower detection limit of the culture system. Owing to the high sensitivity, the percentage of initially positive units is relatively high in our screening system compared to other reported rates of bacterial contamination. Most likely, this is due to the fact that other studies use only one culture bottle, with lower inoculation volumes and shorter culture times. Wagner and Robinette[17] showed that higher inoculation volumes lead to shorter detection times. Our results are very similar to those in Belgium, where a similar screening approach is used.[18,19] When reanalyzing our results according to the system used in Denmark, that is, only the aerobic bottle and 5 days of culture, our result of 0.4 percent initially positive for T5 (with double-swab disinfections and without diversion) is very comparable to the Danish results.[20] Also for apheresis units, the recalculated initially positive rate of 0.1 percent (only aerobic bottle, 5 days culturing) resembles the values reported by other groups[3] taken into account that our inoculated volume is larger.

The selectivity of a screening system should be as high as possible, because false-positive samples result in unnecessary recalls and false-negative samples result in possible transfusion of positive units. For some as yet unknown reason, the rate of negative determination cultures is increased after the introduction of interventions for disinfection and diversion. The fraction of negative determination cultures is 0.047 percent of total tested bottles (two per unit), which is in agreement with data from Belgium and Denmark.[18,20] This would result in a false-positive rate by system failure of about 1 of 2000 cultures. Given that in 9 percent of cases with a negative determination culture for PCs, one of the RBC units contained a microorganism, it remains questionable whether a negative determination culture really should be flagged false-positive. Another possibility would be that in some cases the microorganism still has growth capacity (positive signal in BacT/ALERT), but no colony-forming properties (negative determination culture). This possibility is not hypothetical, because experiments from our group showed that after UV-C illumination the survival of bacteria is much higher (about 1.5 log) when measured in liquid culture, compared to measurement of colony formation on solid media (unpublished results). Based on these results, we intend to implement a follow-up procedure for negative determination cultures, with additional attempts to obtain a subculture.

The effect of introducing a standardized double-swab 70 percent IPA disinfection method showed borderline significance in both univariate and multivariate statistical testing with respect to reduction of bacterial contamination. This is in contrast to literature results. McDonald and colleagues[16] reported a possible reduction of approximately 50 percent by the introduction of double-swab disinfection. These results, however, were obtained with a first swab with IPA and a second swab with iodine tincture, which might explain differences in the results. Lee and coworkers[21] recently also reported a significant reduction of the degree of bacterial contamination of PCs prepared from whole blood by the PLT-rich plasma method, as a result of a changed disinfection method. With respect to skin flora, however, as expected, our new disinfection method has a highly significant reducing effect on the CNS plus group, but no effect on diphtheroids. This can be explained by the fact that diphtheroids especially colonize the interior of the sebaceous glands[22] whereas Gram-positive cocci, including CNS, are more on the skin surface. Surface disinfection will therefore be less adequate to remove diphtheroids, whereas diversion will reduce all kind of skin flora, as found in our study.

The various bacteria species detected in 2 years of bacterial screening of PCs in the Netherlands are classified as skin flora, transient skin flora, or gastrointestinal tract flora, with some very rough subclassification. Within the skin flora, the relatively slowly growing diphtheroids (either anaerobic *Propionibacterium* or aerobic *Corynebacterium*) represent the largest group. These bacteria are usually not considered being a clinical hazard[14,23] but there are some reports describing transfusion-transmitted bacterial infection (TTBI) with *Propionibacterium*.[24,25] Diphtheroids also are among the Gram-positive bacteria that can colonize prosthetic valves and intravascular implants, causing infections that are difficult to treat.[26] Upon prolongation of storage time to 7 days or longer, and without a screening system able to detect these bacteria, one should be aware of transmission of slow-growing bacteria, such as *Propionibacterium* spp. This problem is currently not encountered, because PCs containing these bacteria are generally transfused before reaching the threshold for inducing TTBI in the recipient.

The second largest group are the Gram-positive cocci not being *S. aureus* (CNS plus). These commensal skin flora are not considered very pathogenic either. Gram-positive cocci, however, are a regular cause of TTBI[24,27] but seldom responsible for septic fatalities.[28] The small subgroup of *S. aureus* is the type of skin flora with a high clinical hazard profile[1] but all 13 cases found in our surveillance had a positive culture before the products were released.

The *Bacillus* spp. belongs to the transient skin flora and this group includes some not further determined spore formers. Owing to the etiology of infection (we are only considering transmission via transfusion), no further distinction was made between *B. cereus* (known from food poisoning) and other *Bacillus* spp. The new disinfection method has no effect on the frequency of this group, as expected, because spores are not inactivated by 70 percent IPA. Diversion is expected to result in a reduc-

tion for spore formers, but the observed reduction is not significant, likely because of the low numbers. Because spore formers remain a risk to cause TTBI even with bacterial culturing in place, further research should be focused on improved disinfection also effective in inactivating spores.

Approximately 5 percent of all initial positive samples were determined as normally belonging to the gastrointestinal tract flora, although these bacteria can also be encountered as transient skin flora. The largest subgroup are the anaerobic *Peptostreptococci* spp. (associated with abdominal wound infections) and some *Streptococcus* spp., which are often reported in transfusion-associated sepsis.[28]

The group of Gram-negative rods is very heterogeneous, with *Bacteroides* spp. and *Salmonella* spp. highly likely to have come from the donor's blood circulation. The aerobic Gram-negative rods, although we did not fully determine the species in all cases, are most probably nonfermentative bacilli and are highly likely to have been introduced as transient skin flora. The Gram-negative rods are well known as causative agents for TTBI, especially those cases related to septic fatalities.[1,2,24,25,28] In our study most products positive for the presence of Gram-negative rods were prevented from entering the transfusion chain. In the single case with a transfused RBC unit, the culture turned positive after 5 days.

Among the remaining group, one remarkable microorganism is found, a *Nocordia* spp., well known as transient skin flora present in soil and causing pneumonia, especially in immunocompromised patients or in chronic lung disease.

Despite the absence of a quarantine period, in the Dutch practice more than 90 percent of the units that flagged positive within 2 days could be prevented from being transfused, including the majority of those contaminated with the more dangerous bacteria like Gram-negative bacteria, *B. cereus* and *S. aureus*. Ninety percent of units flagged positive after being released had a positive signal after more than 2 days of culturing. These units were mainly found to be contaminated with diphtheroids, especially *Propionibacterium* spp. and the majority of these units had already been transfused. During the period of the study, two mild transfusion reactions were reported due to transfusion of such units. In general, however, underreporting is noticed in hemovigilance systems, emphasizing the need for improvement. Extension of the storage period of PCs from 5 to 7 days allows for an initial quarantine period as proposed by Munksgaard and colleagues[20] but this will have a very limited impact on the number of PCs already transfused at the time of a positive culture.

In conclusion, it is shown that introduction of diversion and improvement of skin disinfection are effective in reducing the frequency of contaminated blood products. Still, the remaining risk is high enough to warrant screening for bacterial contamination. The screening system in place in the Netherlands proves to be successful in preventing the seriously contaminated PCs from entering the transfusion chain, a similar conclusion to that reported for Belgium[19] with a similar screening system.

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