資料2

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り扱いとされた。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、当該症例にかかる「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「感染症的報告について（目次）」資料は廃止することとする。

議題4について

事務局及び日赤から、供血者からの遡及調査の進捗状況、血液製剤に関する報告事項、献血件数及びHIV抗体・核酸増幅検査陽性件数について説明後、下記のような意見が出された。

（血液製剤に関する報告事項関係）

○ 供血後に何らかの感染症を発症した場合に、血液センター又は医療機関に自分は献血をしたということをお伝え願えると、発症する前に投与された患者さんを特定でき、また、新鮮凍結血漿は6か月の貯留保管をやっているので、医療機関に供給される前に回収することができるということで、今回のＡ型肝炎の事例のように非常にまれな疾患に関しては、供血した後に何らかの異変があった場合に血液センターに連絡をくれるというのが輸血による感染を防ぐ一つの道だと思う。

議題5及び6について

（ＸＭＲＶに関する文献報告関係）

岡田委員から、「ＸＭＲＶに関する文献報告」について報告後、XMRV については、現時点では、血液事業という観点から緊急的な対応は必要ないが、引き続き情報収集を行って、新たな知見等が得られれば、この場で報告していただき、対応を検討することとされた。

（日本赤十字社血液事業本部組織の変更関係）

日赤から、「日本赤十字社血液事業本部組織の変更」について報告があり、下記のような意見が出された。

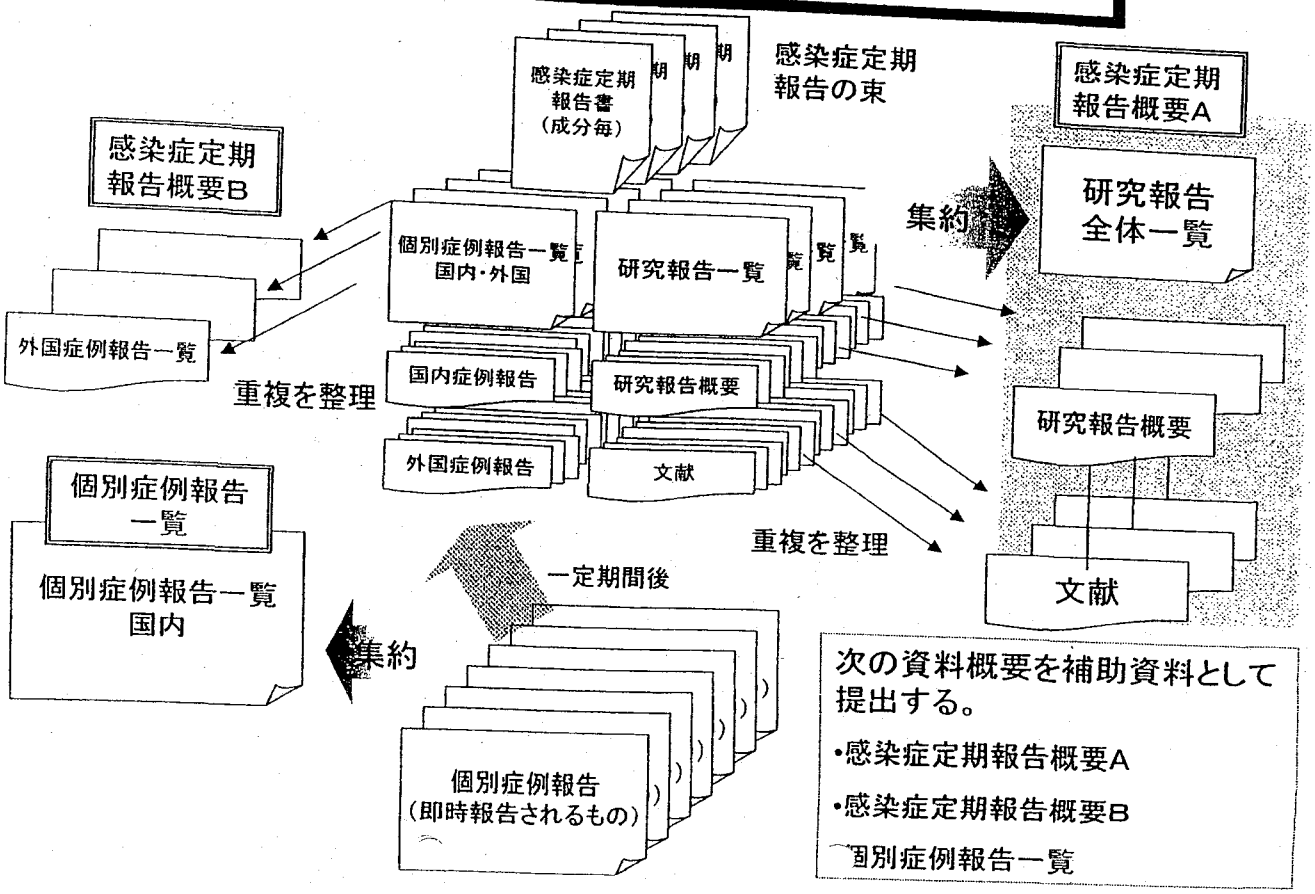
○ 血漿分画部門についても、執行体制としては血液事業本部が統括すると理解しているが、完全に掌握して体制を強化するという意味で、血漿分画部門についても血液事業本部が全面的に経営し、責任もとれる体制へ移行していただきたい。

（フィブリノゲン関係）

事務局から、フィブリノゲン製剤及び血液凝固因子製剤に関する公表等について報告がなされた。

以上

感染症定期報告・感染症個別症例報告の取り扱い



感染症定期報告概要

（平成２２年８月１１日）

平成２２年３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２２年３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、前回の運営委員会において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（2010/3/1～2010/5/31）

血対ID	受理日	番号	感染症(PT)	出典	概要	新出文献No.
100110	2010/4/1	100003	B型肝炎	Transfusion 2009 July; 49; 1314-1320	HBsAg(hepatitis B surface antigen)に陽性を示した供血者とHBV(hepatitis B virus)感染者とのHBVgenotypeを比較するため、HBsAg陽性供血者の遺伝子型を決定した。2006年10月-2007年9月の日本人供血者のデータは日本赤十字社から提供を受け、1887例についてHBVの主な6genotypes(A-F)をELISA(enzyme-linked immunosorbent assay)法によって決定した。HBsAg陽性ドナーについてHBVコア抗原に対するIgM抗体の有無の確認を行った。供血者と患者間で示されたHBVgenotype分布における有意差はC/B遺伝子型比で認められ、この比率は供血者で低く(2.0-3.9)、患者で高かった(5.3-18.2)。また、genotypeBの比率は10歳代の13.8%から増加し、50歳代では42.4%であったが、genotypeC比率は10歳代の83.1%から50歳代の55.1%に減少した。HBcAgに対するIgM抗体およびNAT(nucleic acid test)両者に陽性であるドナーでは、genotypeAおよびBは男性のみであった。日本人供血者におけるHBVgenotypeの年齢特異的な分布は、B/C遺伝子型比に特徴があり、米国もしくは西欧諸国由来であるHBVgenotypeAの性特異的分布は、日本人男性ドナーに観察された。	
100112	2010/3/29	91088	E型肝炎	Emerging Infections Diseases 2009; 15(5): 704-709	E型肝炎ウイルス(HEV)のgenotype3は日本においては不顕性感染とされているが、重篤な肝炎を発症した国内8症例について、強毒性をもたらすHEVの遺伝的特徴を解析するため遺伝子配列を決定した。系統樹解析の結果、いずれも他のgenotype3とは区別され、JIO株と名付けられた固有のクラスターに分類された。このJIO関連ウイルスは他のHEVgenotype3とは異なる18のアミノ酸をコードしており、また、JIOクラスターのヒトHEV株のほぼすべてに共通する置換はヘリカーゼ領域(V239A)に位置し、V239Aはgenotype4では一般的であることから、毒性の増強と関連が示唆された。また、genotype3に属するswJ19株に感染した5匹のブタから遺伝子を解析した結果、同様にヘリカーゼにV239A置換が存在していたことから、JIO関連ウイルスが人獣共通であることが疑われた。	
100115	2010/3/29	91091	E型肝炎	XIVth Regional Congress of the ISBT, Asia; Nov 14-18, 2009; Nagoya (International Society of Blood Transfusion Vox Sanguinis 2009; 97, 17) (2A-S02-03).	HEV(E型肝炎ウイルス)陽性血液の輸血を受けた受血者のルックバック調査から、血液製剤中のHEV高値(>5.4log/bag)がウイルス伝播に関連付けられると考えられた。日本赤十字社では北海道においては、HBV/HCV/HIV-1に加えてHEV NATスクリーニング検査を行っているが、2005年1月-2006年3月には検査結果が判明する前に輸血が行われた場合があった。過去の供血保管検体のルックバック検査により、HEV陽性血液製剤の輸血を受けた13例が判明したが、HEV感染の徴候を示した4例中3例がE型肝炎を発症し、1例は一過性のATL上昇を示した。輸血された4製剤のHEVウイルス量とgenotypeは5.4(G4), 5.5(G3), 5.8(G4), 6.8(G3)log/bagであり、HEV感染を起こさなかった4例へ輸血された4製剤では、<4.4(G3), <4.4(G3), 4.3(G4), 5.5(G3)log/bagであった。genotype4は3より毒性が高い可能性が示唆された。	1

血対ID	受理日	番号	感染症(PT)	出典	概要	新出文献No.
100117	2010/3/29	91093	HIV	FDA/CBER 2009 August Guidance for Industry	2009年8月米国FDAは、ヒト免疫不全ウイルス1型(HIV-1)グループOの感染リスクの高いドナーの管理に関する勧告と題した企業向けガイダンスを発表し、即時適用するよう求めた。 A.HIV-1グループO感染リスクの高い供血者を特定するために問診事項が改定された。以下の質問を供血者問診票(donor history questionnaire)のハイリスク行為についての質問に盛り込むこと。 1.1977年以降、以下の国で生まれたかもしくは居住していたことがあるか:カルメーン、ベナン、中央アフリカ共和国、チャド、コンゴ、赤道ギニア、ケニヤ、カボン、ニジェール、ナイジェリア、セネガル、トーゴ、ザンビア。それはいつか。 2.1977年以降にこれらの国へ渡航歴がある場合、輸血や血液製剤による治療を受けたか。それはいつか。 3.1977年以降にこれらの国で生まれたヒトもしくは居住していたヒトと性的接触を持ったか。それはいつか。 質問のいずれかを肯定した感染の可能性のある供血者を無制限に供血延期とすること。ただし、最後のHIV-1グループOの曝露から1年後に、以下Cの勧告に従って再エントリーを検討できる。 B.HIV-1グループO抗体の検出感度を有するとラベルのIntended Use項に記載された、供血者スクリーニング用の承認済み抗HIV-1/2テストを実施する場合、上記Aの問診を中止してもよい。 C.HIV-1グループO感染リスクの質問への回答に基づき供給延期とされた供給者の再エントリーについて、最後のHIV-1グループOへの曝露から最低でも1年の保留期間を経た後、供給者は以下の場合、再エントリーしてよい。 1.当該供血者の現在の供血時に、HIV-1グループO抗体の検出感度を有するとラベルのIntended Use項に記載された抗HIV-1/2スクリーニングテストの結果、陰性と判明し、かつ 2.当該供血者が全ての供血者適格基準を満たしている。	
100121	2010/3/29	91085	HIV	Nature Medicine 2009; 15(8); 871-872	2001年以降、フランスのレファレンス研究所はHIVの遺伝子多様性を調査しており、2004年に血清検査でHIV陽性であった62歳の女性の血清試料(RBF168)を分析した。この血清は女性がカメルーンからパリに移住した直後に採取された。女性は現在AIDSの症状はない。RBF168からウイルスを分離し、ウイルス遺伝子を解析した結果、RBF168はゴリラのサル免疫不全ウイルス(SIVgor)と最も近縁であった。この新しいウイルスは新しいHIV-1のプロトタイプであると思われるが、HIV-1のグループM,N,Oとは異なり、グループPと命名された。RBF168株が発見される前は、HIVグループOが最もSIVgorに近縁であったが、変異の大きさから現在のSIVgorから直接出現したのではなく、SIVgorのゴリラからヒトへの伝播が起因していると考えられた。これらの結果より、HIVの感染源としてチンパンジーに加えてゴリラが示された。	
100121	2010/4/1	100003	HTLV	51st ASH Annual Meeting and Exposition; 2009 Dec 5-8; New Orleans.	長崎市におけるHTLV-1 (human T-cell lymphotropic virus type 1)の感染率とATL (adult T-cell Leukemia/Lymphoma)の発症率を、(献血者からではなく)2000-2007年に長崎大学病院を受診した患者(10,261名)のHTLV-1抗体検査データおよび長崎県がん登録された長崎市内で診断を受けたATL患者(360名)のデータを評価した。その結果、患者10,261名中HTLV-1抗体陽性者は1,392名(男性:653、女性:739)、陽性率は13.57%であり、女性が有意に高かった。HTLV-1 キャリアの大まかな生涯ATL発症リスクは男性7.29%、女性3.78%と推定され、また、出生年別の抗体陽性率および年間HTLV-1キャリア推定人数からキャリア10万人当たりの年間ATL発症率を推定した。本試験の出生年別HTLV-1抗体陽性率は過去に報告された供血者の陽性率と比べて約50%高く、また、過大評価の可能性はあるが、長崎市において高齢者のHTLV-1キャリアの大規模集団が存在することが示唆された。	2

血対ID	受理日	番号	感染症(PT)	出典	概要	新出文献No.
100121	2010/4/1	100003	HTLV	XIVth Regional Congress of the ISBT, Asia; Nov 14-18, 2009; Nagoya (P-141) (International Society of Blood Transfusion Vax Sanguinis 2009; 97 (suppl.1) 119)	HTLV-1 (Human T-cell lymphotropic virus type 1)抗体陽性供血者(74名)から白血球除去前に採取した血液検体の末梢血単核細胞または凝固血液からDNAサンプルを抽出し、HTLV-1プロウイルス量を調べ、献血者のプロウイルス量の分布と過去のデータから血液検体の80%に感染リスクがあると仮定し、感受性を持つプロウイルス量を推定した。その結果、HTLV-1抗体陽性供血者の血液成分中のHTLV-1感染細胞の最大数(4.9×10^4)は推定される感染性ウイルス量(6×10^5感染細胞)よりかなり低く、血清学的スクリーニングと白血球除去によって輸血感染リスクは事実上排除されていることが示された。	3
100121	2010/4/1	100003	アメリカ・トリパノソーマ症	Transfusion 49 (supplement); 2009 (AABB Annual Meeting and TXPO; 2009 October 24-27)	米国の血液供給におけるT.cruzi (Trypanosoma cruzi)スクリーニングの費用対効果についての報告である。米国の供血血液の75-80%にT.cruziスクリーニング検査が行われており、29,000名当たり1名が陽性と考えられる。本報告では、T.cruziの脅威とその制圧にかかる費用を評価するために、異なるスクリーニング条件下で受血者の仮想コホートを設定し、生涯コストと健康に関する結果を比較するために、病気進行モデリングを用いた。7つの供血者もしくは献血検査の方法を分析し、スクリーニングしない場合と比較した。その結果、モデルにおいて最も影響のあるパラメーターは輸血されたポピュレーションの特徴と関連しており、生存率、健康状態ユーティリティーおよび将来の健康状態の低下率である。T.cruziに関しては、血清陽性率および伝播効率が最も影響している。本分析は、選択的なT.cruziスクリーニングは全数検査とほぼ同等の効果があり、低コストであることを提示している。	4
100121	2010/4/1	100003	アメリカ・トリパノソーマ症	日本感染症学会第58回東日本地方会 2009; 124 041 (2009 October 30-31)	近年、各地医療機関から依頼のあった在日ラテンアメリカ人心疾患患者41名についてジャーガス病原体Trypanosoma cruzi(T.cruzi)血清抗体検査を行った結果、15名が明らかに陽性を示し、ジャーガス病が示唆された。更に抗体陽性者血液からT.cruzi-DNAを検出し、また、血液培養の結果2名からT.cruzi虫体を分離した。慢性の病原体キャリアーが日本に存在することが明らかとなったが、媒介昆虫の存在しない国内において感染経路は二次感染であるため、事前の抗体検査で防ぐことが出来る。	
100139	2010/4/23	100100	ウイルス性脳炎	Emerging Infectious Disease 2009; 15; 1671-1672 (October 2009)	2008年7月、オーストリア東部の山岳地帯で6例が感染したTBE (Tick-born encephalitis) アウトブレイクの調査が行われた。初発患者の羊飼いは、高山牧場地に24日間滞在後、髄膜炎の臨床症状を呈し、TBEV(TBE virus)感染陽性と確定された。患者はダニに咬合された記憶はなく、発症8-11日前に非殺菌のヤギ乳および牛乳から製造された自家製チーズを食べていた。同じチーズを食べた6名中5名がTBE感染と診断され、非感染であった1例はチーズを食べた直後嘔吐していた。チーズはヤギ1頭およびウシ3頭の乳から製造されたが、そのヤギはHIおよび中和抗体検査でTBEV陽性であり、ウシ3頭は抗体陰性であった。また、ホエイおよびヤギ乳を与えられ、同じ牧草地で飼育されていたブタ4頭がTBEV抗体陽性を示した。このアウトブレイクは、中央ヨーロッパ高地におけるTBEの振興と、TBE経口感染の高い効率性を示した。	
100142	2010/4/23	100108	ウイルス感染	Emerg Infect Dis. 2009 Nov;15(11):1830-2.	MAYV (Mayaro virus)はアルファウイルス(Alphavirus)属トガウイルス(Togavirus)科に属し、genotypeDおよびLの2系統が確認されている。2008年2月、ブラジル北部サンタバーバラにある村でMAYVのアウトブレイクが起こり、患者は発疹、発熱および重篤な間接痛が最長7日間続いた。患者血清検体のIgMをELISAで検査した結果、36検体からMAYVに対するIgMが検出された。MAYV分離株3株がgenotypeDと確認され、また、村で捕獲した蚊にはMAYVの主要な媒介蚊であるHaemagogus janthinomysが含まれていた。	5

血対ID	受理日	番号	感染症(PT)	出典	概要	新出文献No.
100145	2010/4/26	100125	ウイルス感染	Eurosurveillance 2009;14(50): pii=19446	2009年8-9月、イタリアにおいて脳髄膜炎と診断された発熱および神経的特徴を伴うびまん性大B細胞性リンパ腫患者に、USUV(Usutu virus)の神経侵襲性感染が認められた最初の報告である。脳脊髄液はUSUV陽性であり、USUVはRT-PCRおよびシーケンスにより血清および血漿で検出された。ウイルス遺伝子のプレメンブレンおよびNS5領域の部分シークエンスはUSUV ViennaおよびBudapestに類似している。	6
100145	2010/4/26	100125	ウイルス感染	Journal of General of Violigy 2009; 90: 2644-2649	1996年、インドケララ州で発生した脳炎アウトブレイクの調査において、蚊(Culex tritaeniorhynchus)のプールからアルボウイルスが分離された。補体結合検査より日本脳炎とウエストナイルウイルスに交差反応を示すアルボウイルスの特徴が示され、アルボウイルス分離株に対する過免疫血清を使用したプラーク減少-中和反応検査の結果、血清は日本脳炎ウイルスでは陽性を示さず、ウエストナイルウイルスで弱陽性であった。このアルボウイルスはバガサウイルス(BAGV)の特徴を示し、脳炎患者の血清は15%(8/53)がBAGV中和抗体陽性を示した。インドからの初のBAGV分離の報告であり、また、人間集団がBAGVに曝露されていることが示唆された。	
100145	2010/4/26	100125	ウイルス感染	ProMED-mail 20090806.2782	2009年8月4日、ブラジルMazagaoで過去3カ月間に657例がオロポーチ熱に感染した事を当局は発表した。このうち29例はIEC(Institute Evandro Chagas)によって確定診断がなされ、この病気の原因はCulicoides属ヌカカによる刺咬であると分かった。症状はデング熱やマラリアに似ており、発熱、頭痛および全身性筋肉痛である。初発例は2009年3月に発生し、4月および5月には報告が激増し、MazagaoのVelhoおよびCarvaoで600を超えた。オロポーチウイルスはブラジルで2番目のアルボウイルス熱の原因ウイルスであり、ブラジルでは過去30年間に約50万人の発熱例が起きている。オロポーチ熱のアウトブレイクはアマゾン地域でのみ報告がある。	
100145	2010/4/20	100079	ウイルス感染	Transfusion Medicine 2009; 19: 213-217	アルブミン溶液、PTC(プロトロンビン複合体)およびFIX(血液凝固第IX因子)からB19V(パルボウイルスB19)およびTTV (torque teno virus)を除去するナノフィルトレーションの可能性を評価した。実験の規模を小さくし、各製剤に各々のウイルスDNA陽性血清を添加し、35nmに続いて15nmのナノフィルトレーションを行った。ウイルス量の測定はリアルタイムPCRによって行われ、15nmナノフィルトレーションは3製剤からB19Vについて4.0log以上の除去能を示し、TTVについては、15および35nmナノフィルトレーションによりアルブミン溶液およびFIXから各々3.0log以上の除去能を示した。また、これらの処理後、ウイルスDNAは測定されなかったが、一方、15nmナノフィルトレーション後のPTCではTTVが検出された。	7
100145	2010/4/26	100125	ウエストナイルウイルス	Emerging Infectious Disease 2009; 15; 1668-1670 (October 2009)	WNV(West Nile virus)感染状況と2003-2008年に供給された米国製血漿由来静注用免疫グロブリン製剤(IGIV)における中和抗体価の関係が調査された。WNVは1999年に米国に持ち込まれたが、2003年にIGIVのWNV中和抗体平均値が顕著に上昇し、米国人口の0.5%がWNVに感染したと推定された。また、米国の人口における既感染者の割合は、毎年0.1%増加し、IGIVの中和抗体価平均値と概ね相関があった。2008年に出荷されたIGIVの中央抗体価は平均21(n=258)であり、NATでWNV感染を確定したヒトから得られた血漿では更に高い抗体価(平均208(n=30))であった。血漿中IgG濃度を補正し、IGIV調整濃度10%と比較すると血漿試料はIGIVより100倍高値であった。この結果は、WNV既感染者は米国人口の1%であると推定したこれまでの報告と一致した。	

血対ID	受理日	番号	感染症(PT)	出典	概要	新出文献No.
100146	2010/4/26	100126	ウエストナイルウイルス	FDA/CBER Guidance for Industry 2009 November	2009年11月、FDAは企業向けガイダンス、「輸血目的の全血および血液成分の供血者からのWNV(West Nile Virus)感染リスクを減じるためのNAT(Nucleic Acid Tests)の使用」を発表した。勧告(Recommendation)の内容は、 A. 検査、ユニット管理および供血者管理:1. 輸血目的の全血および血液成分の供血サンプルにつき、承認されたNAT (MP-NATもしくはID-NAT)を用いてWNVの通年検査を行うこと。WNVの高活動地域ではID-NAT (individual donation)を推奨する。2. MP-NATによる検査の結果、陰性であったミニプールを構成していた検査サンプルのユニットは出荷できる。ミニプールがNAT陽性を示した場合には、ID-NATを用いて各サンプルを検査し、陽性を示したユニットを特定すること。a. すべてのID-NATで陰性であったユニットは出荷できる。b. 個別献血が陽性であった場合、そのユニットは廃棄し、120日間の供血延期とし、該当献血から120日前の期間における製品の回収および貯留を推奨する。3. ID-NATを用いた検査を実施する場合には、A1. 2aおよび2bの手順に従う事を推奨する。 B. MP-NATからID-NATへの切り替え:1. 血液を収集する地域でのWNV活動が高いことを定義する基準を確立し、バリデートすること。2. 血液を集める地域でのWNV活動が高い間、MP-NATからID-NATへ切り替える閾値を設定し、また、活動が収まった際にMP-NATに戻す閾値を設定すること。3. 実行可能になり次第、ただし、閾値到達から48時間以内に、MP-NATからID-NATに切り替える。4.この決定に関するSOPを作成し、従うこと。 C. 検査実施の報告 D. 輸血目的の全血および血液成分の表示	
100146	2010/4/26	100126	黄熱	CDC/MMWR 2009; 59(2): 34-37: 2010 January 22	米国赤十字社はYF (yellow fever)ワクチン接種者には2週間の供血延期を求めているが、2009年4月10日、病院の血液バンク管理者は、血液製剤が供血4日前にYFワクチンを接種した米国軍訓練兵89名から(3月27日に)集められた事に気が付いた。本報告では、供血延期の過失を特定し、輸血に関連したYFワクチンウイルス感染かどうかを決定するために、病院とCDCによって行われた調査を概説している。迅速な回収に関わらず、6ユニットの血液製剤が5人の患者に輸血された。臨床所見や重篤な有害反応を示す検査値の異常は、輸血後一月以内においては、4人の輸血者には見られなかった。5例目は前立腺癌および輸血依存性末期のB細胞性リンパ腫患者であり、ホスピスケアを受け亡くなった。生存者4例のうち3例はYFワクチンウイルスの血清学的反応が検出された。本報告は、輸血に関連したYFワクチンウイルスの感染が起こる根拠を示し、かつ慎重なスクリーニングと直近にワクチン接種したヒトの供血延期の必要性を強調している。	8
100146	2010/4/26	100126	黄熱	CDC/MMWR 2010; 59(5); 130-132 (February 12)	2009年4月、ブラジルにおいて母親が分娩後に黄熱ワクチンを接種し、黄熱ワクチンウイルスが母乳を介して乳児へ伝播したとの報告がなされた。乳児はほぼ母乳のみで育ち、生後23日に抗痙攣薬に治療を要する発作で入院し、髄膜脳炎の治療のため抗菌・ウイルス剤が投与された。乳児のCSF(脳脊髄液)からは17DD黄熱ワクチンウイルスが検出され、血清およびCSFに黄熱特異的なIgM抗体も検出された。調査の結果、乳児は母乳を介した黄熱ワクチンウイルス感染と特定され、黄熱ウイルスの曝露が避けされないもしくは延期できない場合を除き、授乳中の女性への黄熱ワクチン接種は行うべきではない。	9

血対ID	受理日	番号	感染症(PT)	出典	概要	新出文献No.
100146	2010/5/20	100158	感染	Google News 2009 December 18	2009年12月18日、臓器提供者から少なくとも1人の臓器移植者に極めて珍しい感染が認められ、初のアメーバ(Balamuthia mandrillaris)のヒトーヒト感染が報じられた。11月にUMMC (University of Mississippi Medical Center)で神経障害で亡くなった患者から臓器提供を受けた4例のうち、2例は重症(それ以外は無症状)であり、CDCは1例にBalamuthia mandrillarisを確認した。この微少寄生虫は土壌で発見され、ヒト、ウマ、イヌ、ヒツジおよび霊長類に脳炎を引き起こす。免疫抑制状態にある臓器移植患者では危険な寄生虫である。ヒト感染は極めて珍しく、1990年の発見後、世界で150例のみが報告されている。	
100146	2010/4/26	100126	クラミジア	Infection, Genetics and Evolution 9; 1240-1247; 2009	フランスにおいて、家禽屠殺場の従業員3例に非定型肺炎がおき、10の養鶏場で疫学調査が行われた。25調査群中14群にクラミジア関連因子が検出され、オウム病クラミジア(Chlamydophila psittaci)が検出されたのは陽性群中1群のみであり、これまでに分類されていない新規クラミドフィラ属の存在が明らかとなった。更に6陽性群からクラミジア菌を分離した結果、すべてのサンプルにおいて16s rRNA遺伝子配列の相同性がほぼ一致し、また、現在認められているクラミドフィラ属の株とは異なるが、同属であることが示された。一方、ompA遺伝子の配列は分離菌間で大きく異なった。各農場はこの新しいクラミジアの異なる株によって感染されたことが示された。	10
100147	2010/4/26	100127	細菌感染	Transfusion 49; 2152-2157; 2009 October	日本において初流血除去導入前および導入後の血小板濃厚液(PC)の細菌汚染頻度を調査した。日本赤十字社が供給する、初流血除去導入前および導入後の有効期限切れPCを用い、保存から4日以上後に血小板検体をサンプリングし、好気性および嫌気性ボトル双方に10mL量を接種した。その結果、細菌感染は初流血除去導入前後で0.17%(36/21,786)から0.05%(11/21,783)に減少し(減少率71%)、臨床的に重大な細菌の汚染件数は4件(0.018%)であった。本結果より、初流血除去の効果は細菌汚染頻度において顕著であった。また、細菌汚染頻度は西欧諸国と同等であり、培養スクリーニング検査は非実施であるにも関わらず、日本ではPC輸血後の敗血症反応の発現頻度が低いのは、日本でのPCの保存期間が72時間と短い事が理由として考えられ、この重要性を示唆する結果であった。	11
100148	2010/4/26	100128	チクングニヤウイルス感染	CDC/Traveler's Health 2010 April 7	2010年4月7日現在のアジアおよびインド洋におけるチクングニヤ熱のアウトブレイクについてCDCが報告した。当該地域における最近のチクングニヤ活動の高い地域は、インドネシア、タイおよびマレーシアであり、各国のアウトブレイク状況が示された。医者へのアドバイスとして、チクングニヤはマラリアやデングと発熱・悪寒・全身筋肉痛などの症状が似ているが、チクングニヤにおいては、急性期後に関節痛や関節炎が長引き、リウマチの検査が必要かと思われることがある。また、当該疾病が報告されている地域への渡航者に向け、露出している肌への虫除けの使用など、アドバイスも掲載されている。	12
100148	2010/4/26	100128	チクングニヤウイルス感染	日本感染症学会第58回東日本地方会 2009; 124 041 (2009 October 30-31)	2009年5-6月、東南アジアから帰国後間接痛を主訴に来院した3例はチクングニヤウイルスIgM抗体および中和抗体陽性であり、血清学的にCHIKF(Chikungunya fever)と診断された。3例はそれぞれインドネシア・スマトラ島、インドネシア・ジャワ島もしくはマレーシア・クアラルンプール郊外に渡航し、いずれも現地で発熱および関節痛が出現した。解熱したが帰国後も関節痛は持続し、受診に至った。	

血対ID	受理日	番号	感染症(PT)	出典	概要	新出文献No.
100152	2010/4/27	100140	デング熱	AABB Annual Meeting and TXPO; 2009 Oct. 24-27; New Orleans (Transfusion 2009; 49 suppl. S66-030Q)	プエルトリコ(PR)では2007年に2005年のアウトブレイクより更に大きなデング熱アウトブレイクが起き、この間の供血者におけるデング熱ウイルス血症の割合について調査が行われた。調査用に保存された供血サンプルは米国およびPRで輸血されたユニットであり、TMA (transcription mediated amplification assay)で初回陽性(Initially reactive:IR)であったサンプルは再検査が行われ、再度陽性(repeat reactive:RR)を示した場合には追加の検査が行われ確定された。検査が実施された15,350サンプルのうちIRは28、RRは25サンプルであり、陽性の割合は1:614であった。また、米国に輸出されたデング熱陽性供血者は12例(1:533)、PRでは13例(1:689)であった。RRサンプル中約半数はIgM陰性で高力価ウイルス血症を示し、細胞培養検査において感染性が認められた。デング熱が流行している間は供血者のスクリーニングの措置が検討されるべきである。	13
100157	2010/5/6	100150	デング熱	ProMED-mail 20090831.3065	ベトナムハノイ市では、デング熱症例が深刻な増加を示しており、2009年初から8月下旬までに2500症例が報告され、これは2008年の同時期と比べて10倍以上であった。また、ホーチンミン市ではデング熱症例数の急増はないものの、多くの患者が重症化しており、死亡例も多くなっている。同市の第一小児病院は、毎日20-25人がデング熱症例のため来院しており、小児のデング熱症例は、感染後1-2日は手足口病やH1N1インフルエンザとの判別が難しいためデング熱への警戒をゆるめることがあるが、小児は死に至ることがあると注意喚起した。	
100157	2010/5/6	100150	バベシア症	ABC Newsletter #41: 4-5; 2009 November 13	ARC (American Red Cross)はバベシア症の拡大報告を受け、米国7州での供血検査の実施を提案している。近くTransfusion誌に掲載予定の研究報告3報では、1.供血血液のBabesia microtiに対するIgG抗体を調査した結果、コネチカット州およびマサチューセッツ州で広範囲な拡大が確認され、2. ロードアイランド州における輸血を介した感染の広がりを特定し、3. 2005-2007年にARCのHemovigilance Programに報告されたTTB (transfusion-transmitted babesiosis)症例の分析が成された。これらの報告は、バベシア症およびTTBが増加している危険性への懸念が強調されており、ARCは感染地域での供血血液検査を行う2つの提案を作成した。まずコネチカット州でIFA(immunofluoresence assay)による(全血献血された)供血血液検査、陽性供血者の供血延期などを行い、この結果次第ではあるが、他の6州でも検査範囲を広げる予定である。バベシア症はIxodes属のマダニによって伝播し、大部分の感染者は無症候か軽症で何ヶ月も続く可能性がある。現在、FDAが認可した検査方法はなく、寄生虫保有者が供血した場合、受血者への輸血を介した感染の可能性がある。	14
100157	2010/5/6	100150	パルボウイルス	Blood 114(17): 3677-3683; 2009 October 22	成分輸血によるパルボウイルスB19感染を評価するために、供血者と受血者の関係が既知である保存血液検体およびB19V DNA定量可能なPCRを用いてB19V感受性(抗B19V IgG陰性)である受血者のB19V感染について調査した。輸血前B19VのIgG抗体陽性率が78%である手術歴のある患者群に輸血が行われた105名の供血者から成る112のB19V DNA陽性である成分輸血について評価した。B19V DNAが10^6IU/mL以下である成分輸血を受けた受血者24名には感染が認められず、B19V DNAが10^{10}IU/mL以上である成分輸血を受けた輸血前の抗体陽性である受血者1名に既往反応が認められた。B19V DNAが10^6IU/mL以下である成分輸血からは感染は起こらない、もしくは、まれであることが示唆され、献血の定常的なスクリーニングにB19V DNAのNAT検査は不要であることを指示している。	15

血対ID	受理日	番号	感染症(PT)	出典	概要	新出文献No.
100157	2010/5/6	100150	ボリビア出血熱	Emerging Infectious Disease 2009; 15: 1526-1528 (September 2009)	BHF(Bolivian hemorrhagic fever)は1959年にボリビア東部でのアウトブレイク発生時に初めて報告され、2007年2-3月、ボリビアで少なくとも20例(死亡3例)のBHF疑い例が報告された。2008年2月には少なくとも200例(死亡12例)の疑い例が報告され、19症例の血清を間接免疫蛍光法およびPCRを用いて検査した。その結果、アレナウイルス5株が分離され、ウイルスRNAの遺伝子配列の結果、マチュポウイルスを確認し、8つの主要な系統に分類された。その後も、マチュポウイルスは孤発症例やボリビアでのBHFアウトブレイクの原因となっているが、5例(死亡3例)の農業従事者である患者については、5例ともBHF感染歴のある患者からの血漿成分輸血を受けたが、3例は死亡した。病状が重篤化する前に、マチュポウイルスによって免疫が惹起された血漿を投与することが生存率を高くする。	
			マラリア	Clinical Infection Diseases 2009; 49: 852-860	ヒトにおけるPlasmodium knowlesi感染の臨床的な特徴および検査結果を調べる目的で、急性P. knowlesi感染患者の背景と経過について系統的に調べ、2006年7月-2008年2月に、Kapit病院でPCRにより急性マラリアと確定された、治療歴の無い非妊娠成人から臨床データおよび検査結果を収集した。152例のうち、P.knowlesi、P.falciparumもしくはP.vivaxに感染した症例は107(70%)、24(16%)および21(14%)であり、非特異的発熱症状のあるP.knowlesi感染患者の入院時寄生虫値の中央値は1387parasite/ulであり、全例が血小板減少を示した。ほとんどのP.knowlesi感染患者には合併症はなく、クロロキンおよびプリマキン治療で治癒した。WHOの熱帯性マラリアの判断基準により7人は重症であった。入院時のP.knowlesi寄生虫血症は呼吸困難の独立した決定因子であり、入院時の血清クレアチニンレベル、血清ビリルビンおよび血小板数と同様であった。2例のP.knowlesi感染患者が死亡し、死亡率は1.8%(95%信頼区間、0.2-6.6%)であった。P.knowlesiは広範囲の疾病を引き起こすが、多くの場合合併症伴わず、治療に速やかに反応し、約10人に1人が死亡を伴う合併症となる。	
100157	2010/5/6	100150	レトロウイルス(XMRV)	PLoS ONE 5(1): e8519. doi:10.1371/journal.pone.0008519	2009年10月に米国で報告されたCFS (Chronic Fatigue Syndrome)患者101名のうち68名に(これまで前立腺癌との関連が示唆されている)新規ガンマレトロウイルスXRMV (xenotropic murine leukemia virus-related virus)を検出した知見を受け、英国においてCFS患者186名の血液試料からDNAを抽出し、nestedPCRによりXMRVのスクリーニングが行われた。DNAの質の確認には細胞内β-グロブリン遺伝子が増幅され、またネガティブコントロール(水)およびポジティブコントロール(XMRV感染分子クローン)も実施された。186例試料すべてにおいてβ-グロブリン遺伝子は増幅されたが、XMRVおよび(関連性の高い)MLV (murine leukemia virus)は検出されなかった。英国においてはCFSとXMRVの関連を示す証拠は見つからなかったが、北米と欧州ではXMRV有病率に集団差がある可能性があり、(これまでに)二つの米国グループは前立腺癌組織にXMRVを検出し、二つの欧州の研究では検出されなかった事実もこの可能性を説明するかもしれない。	16
100158	2010/5/6	100151	レトロウイルス(XMRV)	Science 2009; 326: 585-588	CFS(Chronic Fatigue Syndrome)患者の血液細胞に感染性レトロウイルスXRMV(xenotropic murine leukemia virus-related virus)を検出した。CFSは原因不明の衰弱していく疾患で、世界中で1700万人が罹患していると推定されている。CFS患者の末梢血単核球を調べた結果、ヒトgammaretrovirusであるXMRVのDNAが、患者101例中68例(67%)に検出され、健康対照者では218例中8例(3.7%)であった。細胞培養の結果、患者由来のXMRVは感染性があり、ウイルスの細胞を介したおよび無細胞性感染のいずれも可能性が示された。CFS患者由来の活性化PBMC、B細胞、T細胞に曝露した後、非感染初代培養リンパ球および指標細胞培養系には二次感染が認められた。これらの結果は、XMRVがCFSの病原性における要因となる可能性を示唆した。	

血対ID	受理日	番号	感染症(PT)	出典	概要	新出文献No.
100133	2010/3/18	91020	インフルエンザ	CDC FluView 2009-2010 Influenza Season Week 1 ending January 9, 2010	米国CDCのインフルエンザ部門からの週刊報告であり、week 1 (2010年1月3-9日)における国内インフルエンザ活動は減少し続けている事が報告された。ヒトにおける新規インフルエンザAウイルス感染1例の報告もあり、ウイルスはブタインフルエンザA (H3N2)と確認され、2009年11月に調査が行われた。感染者のブタとの接触は明らかではなく、ヒト-ヒト感染を疑う証拠は認められていない。	17
100136	2010/4/23	100097	インフルエンザ	日本ウイルス学会第57回学術集会 1P074 (p.355)	日本で採血された血漿を原料として製造された静注用グロブリン製剤(IVIG)にClassical Swine Influenza A(H1N1) virusおよびInfluenza A(H1N1) pdm virusに反応する抗体が含まれているか調べ、ドナーが免疫を獲得している可能性について検討した。その結果、IVIGにブタおよび新型ウイルスに対するHIおよびNT活性がそれぞれ8倍、64倍と認められ、日本において、ある程度の率でInfluenza A(H1N1) pdm virusに反応する抗体を保有するドナーが存在すると推測された。	
100147	2010/4/26	100127	新型インフルエンザ(H1N1)	ABC Newsletter #38. 2009 Oct 23; 13-14.	欧州連合の血液規制委員会 (Blood Regulatory Committee)は、H1N1インフルエンザパンデミックが血液供給に不足をもたらすとの報告があることから、血液供給を十分確保するために2つの規制を緩和する事を検討している。欧州各国の代表は、深刻なパンデミックにより10-15%の輸血用血液が不足となる事を懸念している。委員会はEBA (European Blood alliance)、欧州内のassociation of national suppliers and regional alliancesおよび各国規制機関(Competent Authority)に出席を依頼し、安定供給を維持するためにどの規制を緩和するか検討した。インフルエンザ様症状回復後の献血延期期間の短縮(14日から7日もしくは5日へ)およびヘモグロビン値の基準の緩和(女性:12.5から12g/dLへ、男性:13.5から13g/dLへ)を検討している。	18
100147	2010/4/26	100127	新型インフルエンザ(H1N1)	Clinical Infectious Diseases 2010; 672-678	重症パンデミック2009インフルエンザA(H1N1)ウイルス感染とイムノグロブリンG2(IgG2)欠損との関連を調査するため、H1N1感染患者集団におけるIgGサブクラスのレベルを調べた。H1N1感染患者は、重症・中程度・健康妊婦に分類し、血液サンプルを比較した。低アルブミン血症、貧血、総IgG・IgG1・IgG2レベル低下については、統計学的に有意に重症H1N1感染と関連が認められた。多変量解析後にも有意差が見られたのは低アルブミン血症と平均IgG2レベル低下であった。IgG2欠損患者を平均90日間追跡調査したところ、低アルブミン血症はほとんどの患者で解消していたが、IgG2欠損は解消しなかった。健康妊婦では軽度のIgG1/IgG2低下が認められたが、H1N1感染妊婦ではIgG2レベルが有意に低かった。	19
100147	2010/4/26	100127	新型インフルエンザ(H1N1)	Emerging Infectious Diseases 2010; 16 (4); 722-723 April 2010	日本血液センターは献血後にインフルエンザAパンデミック(H1N1)2009感染の可能性のある血液製剤につき、NAT(nucleic acid amplification technology)を用いてウイルス遺伝子を検査した。献血後7日以内にインフルエンザの症状を示した96人の供血者から血漿96検体および赤血球67検体を検査した結果、パンデミック(H1N1)2009ウイルスはいずれの検体でも検出されなかった。パンデミク(H1N1)2009ウイルスにおけるウイルス血症は非常に低くNATで検出されない、もしくはウイルス血症を確認できない程短い期間であることが示唆される。	20

血対ID	受理日	番号	感染症(PT)	出典	概要	新出文献No.
100148	2010/4/26	100128	新型インフルエンザ(H1N1)	Eurosurveillance 2010;15(9): pii=19498	2009-2010年のノルウェイにおいて、2009パンデミックインフルエンザA(H1N1)ウイルスのhaemagglutinin HA1に変異のあるD222Gは死亡および重篤症例に頻繁に認められたが、中程度の症例では事実上認められなかった。この差異は統計的に有意であり、本結果は、この変異と臨床症状間における因果関係と一致している。本報告は、重症例の転帰と関係のあるパンデミックウイルスにおける変異の特定を行った最初の報告である。一方、非重篤症例においても、実際に変異ウイルスが非常に低い頻度で流行しているかを判断するために更なる軽度の症例数が必要である。	21
100148	2010/3/1	90967	新型インフルエンザ(H1N1)	農林水産省 新型インフルエンザに関する報道発表資料 2009 October 21	2009年10月21日、農林水産省は大阪府の養豚農場のブタから分離されたウイルスが新型インフルエンザであることを発表し、当該農場に対し、臨床検査および遺伝子検査により異常がないことを確認するまで飼育ブタの移動を自粛するよう要請した。(独)農研機構動物衛生研究所がHおよびN亜型検査(遺伝子解析)を実施した結果、本ウイルスはH1N1亜型であり、新型インフルエンザと同一である事を確認した。	
100166	2010/5/27	100182	新型インフルエンザ(H1N1)	FDA/CBER Guidance for Industry (DRAFT) 2009 November	2009年11月、FDAは企業向けガイダンス案、「パンデミック(H1N1)2009ウイルスに対応した供血者の適合性、血液製剤の安全性および血液供給の保全について評価するための勧告」を発表した。勧告(Recommendation)の内容は、 A.交代要員の教育 B.供血者の適合性 C.供血延期:使用可能なデータは、パンデミック(H1N1)2009インフルエンザ感染又は疑いのある患者、もしくはインフルエンザ様症状を呈する患者との接触のあった場合に供血延期を求めていない。供血者は供血日に健康であることを確保するため、パンデミック(H1N1)2009インフルエンザ感染又は疑いのある供血者は、解熱剤の利用なく解熱し、無症状となってから少なくとも24時間の供血延期をすること。使用可能なデータは、パンデミック(H1N1)2009ウイルスに対する生もしくは不活化インフルエンザワクチンを接種した後、もしくは、予防目的で抗ウイルス薬であるオセルタミビルおよびザナミビルを使用した後の供血延期を求めていない。しかし、パンデミック(H1N1)2009インフルエンザ感染又は疑いのために抗ウイルス薬を服用した供血者は上述と同様少なくとも24時間の供血延期をすること。血液製剤の管理:供血後48時間以内にパンデミック(H1N1)2009の感染又は疑いがある、もしくは、インフルエンザ様症状を呈したという供血後の情報を受けた際には、メディカルドクターはSOP(標準操作手順書)に従い供血製品の安全性を評価すること。なお、この勧告は、全血液および輸血に適用される血液成分に適用される。	
100168	2010/5/27	100184	新型インフルエンザ(H1N1)	The Canadian Press 2009 September 16	オーストラリアの研究グループは新型A1N1ウイルスに感染し重症となった妊婦では、ウイルスと戦い、体がワクチンに反応する助けとなる、特定の抗体が低値である事を発見した。ICUで治療中のブタインフルエンザ感染患者すべての抗体レベルを個々のサブタイプまで調べた結果、IgG2のレベルが低値であった。妊娠女性についてのみ調べた結果であるが、このIgG2欠損が、ほとんどの人はインフルエンザ症状のみで治癒するが少数例は危篤となる理由が説明できる可能性がある。	
100168	2010/5/27	100184	鳥インフルエンザ	Virus Genes. 2009 Aug;39(1):76-80.	トリインフルエンザH5N1ウイルスの感染者における組織分布および肺以外の臓器での複製能を調査するため、感染患者の剖検を実施した。ウイルス量は脾臓に比べて肺で最も多く、心臓・肝臓・腎臓・大小腸または脳ではウイルスは検出されなかった。また、ウイルス量は右肺に比べて左肺でより多く検出され、左肺の病理組織により重篤な損傷が認められた結果と一致し、また、左肺組織には+/-鎖双方のウイルスRNAが存在した。一方、低ウイルス量であった脾臓には+鎖ウイルスRNAは認められず、循環血液などによって運ばれた可能性が示唆される。	22

血対ID	受理日	番号	感染症(PT)	出典	概要	新出文献No.
100109	2010/3/29	91085	BSE	PLoSONE 2009; 4; E6175	魚類におけるTSE(transmissible spongiform encephalopathies)発症についての知見を得るため、gilthead sea beam(sparus aurata:ヨーロッパヘダイ)にBSE感染ウシもしくはスクレイピー感染ヒツジのホモジネートを経口投与した。魚に臨床症状は現れなかったが、投与2年後、魚の脳は神経変性の徴候と抗タイPrP抗体に陽性を示す沈着物の蓄積が認められた。非感染動物由来の脳を投与された対照群はこのような徴候はなかった。TSE感染脳よりもBSE感染脳投与された魚に多数のプロテアーゼK抵抗性沈着物が急速に現れ、アミロイド様成分と一致した。公衆衛生上の潜在的なリスクの懸念が高まる。	
100110	2010/4/23	100101	BSE	Schweiz Tierheikd 151; 433-436	スイスにおいて、BSE (bovine spongiform encephalopathy)を発症したウシの仔(グループA)に、血漿中PrPres (protease-resistant prion protein)が産生されているかを調査し、また、健常ウシ(グループB)と陽性頻度を比較した。グループAはBSEを発症したウシの仔181頭、グループBは2001-2006年にBSE症例のないスイスの健常ウシ240頭で成っている。すべての血漿はAlicon PrioTrapを用いて評価され、PrPresの生前検査が行われた。仔181頭中29(16.1%)は血漿中PrPres陽性であり、母ウシがBSEを発症する1年以内前に生まれた仔は、母ウシ発症の1年以上前に生まれた仔より、PrPres陽性血漿の頻度は顕著に高く、健常ウシでは240頭中10(4.2%)であった。PrPres はウシ血液中に検出可能であり、健常ウシ群よりBSE発症ウシの仔により高頻度に検出された。	23
100147	2010/4/26	100127	クロイツフェルト・ヤコブ病	Neuropathology 29(5); 625-31; 2009 October	日本CJDサーベイランス委員会 (CJD Surveillance Committee)により、最近の9年間で登録された患者に行われた医療(外科処置、脳神経外科処置、眼科手術および輸血)が調査された。孤発性CJD (sporadic CJD)753名と対照被験者210名から成る症例対照試験において、プリオン病がsCJD発症前に調査対象の医療を介して伝播したことを示すエビデンスを見出せなかった。これまでに報告された症例対照試験のレビューにおいて、輸血がCJDの有意なリスク因子であることが示された事はなく、本試験も同様の結果であった。本試験において、sCJD発症後に手術を受けたsCJD患者の4.5% はsCJD発症後に手術(0.8%が脳神経外科処置および1.9%が眼科手術)を受けており、sCJD発症後ですら手術(脳神経外科処置および眼科手術を含む)を受けた患者がいる事実は、医療処置を介したプリオン伝播の可能性を除外できないことを示唆している。	24
100121	2010/3/29	91089	異型クロイツフェルト・ヤコブ病	AABB Weekly Report 15(39) 2009 October 22	米国AABBのTTD(Transfusion Transmitted Diseases) CommitteeがAABBのBoard of Directorへ提出したTSE (Transmissible Spongiform Encephalopathies)の現状と輸血の安全性に関する報告書である。これまで、vCJDを発症した3例からの輸血によってvCJDプリオンが伝播した4例の報告がある。そのうち、3例はvCJD発症に至り、他の要因で亡くなった1例は脾臓およびリンパ節からvCJDプリオンが検出されたが、vCJDの兆候を示さなかった。その患者はプリオン遺伝子の129番目コドンがヘテロ(MV)であった。また、vCJDを発症したドナーから血漿分画製剤を投与された患者に、vCJDプリオンが検出されたが、この患者もvCJDの兆候はなかった。米国FDAは2009年6月に、米国内供給された血漿製剤給血者のvCJD伝播のリスクに関する新しいモデルをTSE Advisory Committeeで発表したが、最大推定リスクは1/12,000のままであり、米国患者のリスクは「極めて低い」としている。しかし、MVもしくはVV遺伝子型である無症候患者から病原性プリオンが検出されたことから、非MM遺伝子型患者にvCJD症状が現れるか、非MM遺伝子型患者はvCJDプリオンの感染キャリアーとなるかについて解決が待たれる。	

血対ID	受理日	番号	感染症(PT)	出典	概要	新出文献No.
100130	2010/4/20	100080	異型クロイツフェルト・ヤコブ病	AABB Weekly Report 16 (10) 2010 March 12	NIH (National Institute of Health)の研究者はマウスにおいてプリオン関連の障害の特徴であるスポンジ様の脳損傷を引き起こさないプリオン病の新しい形状を報告した。この新しいプリオン病は脳動脈を破壊するアルツハイマー病と関連した脳アミロイド血管障害と似ている。本研究は、プリオン病の徴候が多数発現したが、プリオン病を代表するニューロン内外のスポンジ様の穴は観察されず、マウス脳は動脈、静脈および毛細血管の損傷により血管外で捕捉されたプリオン蛋白プラークが蓄積されていた。	25
100133	2010/4/23	100094	異型クロイツフェルト・ヤコブ病	Haemophilia 2010; 1-9	英国において、vCJD(variant Creutzfeldt-Jakob disease)のリスクが高いと考えられるが、神経症状を呈しない血友病患者17例(剖検11例および生検7例)につき、疾病と関連のあるPrPres(protease-resistant prion protein)の検出を検討した。1剖検の脾臓組織はウエスタンブロット解析の結果、PrPres強陽性を示した。組織の由来元である73歳男性患者は、神経症状を発現しなかったが、プリオン蛋白のコドン129番目がヘテロ型(メチオニン/バリン)であり、vCJD感染ドナーからの供血を含む(もしくは不明な)第Ⅷ因子製剤を投与されていた。他にも赤血球製剤投与や外科手術、内視鏡を経験しており、諸要因を介する関連リスクのうち、英国血漿製剤投与からの感染が最も示唆される。	26
100158	2010/5/6	100151	異型クロイツフェルト・ヤコブ病	Biologicals 2009; Available Online 1-3 2009 November 19	感染性プリオンタンパクの除去を目的とした孔径15nmのウイルス除去フィルターの評価を行った。フィルター濾過前にアンチトロンビンサンプルに異なる二つの方法で調整されたプリオンサンプルをスパイクした。動物を用いたバイオアッセイによるLRF(log reduction factor)は、2回の独立した濾過において≧4.72および4.00であった。しかしながら、感染性は15nmフィルター濾過したサンプルの超遠心分離後の沈殿物と上清の両者に検出され、完全除去は困難であることが示された。本データは、感染性プリオンタンパクの一定量は直径15nmより小さい、かつ(もしくは)可溶性であるとの結論を提示している。	27
100158	2010/5/6	100151	異型クロイツフェルト・ヤコブ病	FDA/CBER 2009 September 7	FDAのCBERは、米国承認血漿由来第Ⅷ因子製品(pdFⅧ)によるvCJD(variant Creutzfeldt-Jakob disease)リスクの可能性についての概要を公表し、要点として以下が示された。 ○近年、米国承認pdFⅧ製品を投与された血友病AおよびVon Willebrand病患者にvCJDが発病するリスクに関して疑問が提起されている。 ○リスク評価の結果、FDA、CDCおよびNIHも含めて米国PHS(Public Health Service)は、米国承認pdFⅧ製品を投与された血友病AおよびVon Willebrand病患者へのvCJDリスクは、はっきりとは分からないが、極めて小さい可能性が最も考えられる。第Ⅸ因子を含めた他の血漿由来製品によるvCJDリスクは同程度小さいもしくはより小さい可能性が最も考えられる。 ○新しい情報を得るには、Hemophilia Treatment Centerの血友病もしくはVon Willebrandにおける専門家に尋ねること。	
100159	2010/5/20	100158	異型クロイツフェルト・ヤコブ病	Lancet 2009; 374; 2128-2128	2008年6月、30代男性が13ヵ月に亘る人格変化、進行性不安定および知能低下にて入院し、2009年1月に死亡した。病歴において輸血および他人からの臓器移植を受けていなかった。、患者PRNP(prion protein gene)のコドン129には疾病と関連が知られている変異はなく、ヘテロ接合体であった。vCJD (variant Creutzfeldt-Jakob)との診断は、臨床症状と進行、MRI所見、他の診断を排除した結果なされ、また、孤発性CJDは不適当と判断された。ヒトPRNPのコドン129における多型がプリオン病の大きな感受性因子となっており、これまでのvCJDでは全症例がメチオニンホモ接合体であった。	28

血対ID	受理日	番号	感染症(PT)	出典	概要	新出文献No.
100164	2010/5/27	100180	異型クロイツフェルト・ヤコブ病	ProMED-mail 20100107.0076 [1] (UK: National CJD Surveillance Unit - monthly statistics as of 5 Jan 2010)	英国CJDサーベイランスユニットの月間統計によると、2010年1月5日現在、2009年の確定もしくは疑いvCJD患者の総数は170名(死亡:166名、生存:4名)である。2009年に2名のvCJD新規症例が登録されたが、英国におけるvCJDアウトブレイクは減少しているとする見解と一致している。	29
100164	2010/5/27	100180	異型クロイツフェルト・ヤコブ病	UK Department of Health, SaBTO (Summary of the 8th Meeting); 2009 October 27	英国SaBTO(Advisory Committee on the Safety of Blood, Tissue and Organs)の第8回会議(2009年10月27日開催)の要旨が示されている。プリオンフィルターについて、プリオンフィルター処理赤血球の安全性を評価する臨床試験(PRISM trail)および同製剤の有効性評価からの新しいデータがvCJDワーキンググループから報告された。臨床試験の初期結果は有望であったが、この試験には完了までに時間がかかる事が分かり、動物を使用した内部の有効性試験からデータが得られるのは2014年になる。これらの情報と分析から、委員会は1. 本フィルターが感染を低減する十分な証拠が現在はあるとし、2. PRISM臨床試験の完了を条件とし、フィルター処理赤血球は1996年1月1日以降に生まれたヒトへの提供を推奨する。	30
100165	2010/3/29	91081	異型クロイツフェルト・ヤコブ病	Vox Sanguinis 2009; 96; 270	1995年から3回/週でIVIG治療を受けていた61歳女性は、1997年1月～1998年2月の期間に、後にvCJDを発症した供血者由来の製剤を使用していた。この女性の死亡後、剖検により脾臓、リンパ節、脳内のプリオン蛋白を検査したが、検出されなかった。	
100165	2010/3/29	91081	異型クロイツフェルト・ヤコブ病	Vox Sanguinis 2009; 97(3); 207-210	英国ではvCJD(variant Creutzfeldt-Jakob disease)症例における血漿分画製剤の投与歴を明らかにするため、英国NCJDSU (National CJD Surveillance Unit)が患者の親戚や診療機関および病院を通して集めた記録の調査が行われた。NCJDSUでは問い合わせのあった全vCJD症例につき、リスク要因となる情報収集を行っている。その結果、168例の英国内vCJD症例のうち9例がのべ12回血漿分画製剤の投与を受けていた(1例はvCJDリスクが起きる前の1970年であり、それ以外は1989-1998年であった)。英国CJD Incident Panelのリスク評価基準によると、11については低リスク製品であり、1つは低もしくは中程度のリスクであった。今日までの英国内vCJD症例はいずれに関しても血漿分画製剤投与による感染ではないと考えられたが、今後、vCJDを発症する可能性は排除されない。	

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
				2009. 12. 20	該当なし	
一般的名称	人血清アルブミン		研究報告の公表状況	Sato S, Matsubayashi K, Sakata H, Takeda H, Kato T, Ikeda H. XIVth Regional Congress of the ISBT, Asia; Nov 14-18, 2009; Nagoya.	公表国 日本	
販売名(企業名)	赤十字アルブミン20(日本赤十字社) 赤十字アルブミン25(日本赤十字社) 赤十字アルブミン20%静注4g/20mL(日本赤十字社) 赤十字アルブミン20%静注10g/50mL(日本赤十字社) 赤十字アルブミン25%静注12.5g/50mL(日本赤十字社)					

研究報告の概要

○E型肝炎ウイルス(HEV)陽性血液の輸血を受けた受血者のルックバック調査

目的:2004年までに輸血によるHEV感染症例を少なくとも2例観察した。それ以来、日本赤十字社では北海道において、HBV/HCV/HIV-1に加えて、HEV NATスクリーニング検査を行っている。本試験の目的は、HEV陽性血液の輸血を受けた受血者のルックバック検査を行うことにより、輸血によるHEV伝播が生じる要因を検討することである。

材料および方法:2005年1月～2006年3月まで、全ての供血者検体について、HEV-NATスクリーニング検査を行った。しかし、一部の血液製剤は検査結果が判明する前に輸血されていた。2006年4月以降、北海道で採血された血液製剤はHEV-NATスクリーニング後に供給されている。過去の供血保管検体のルックバック検査で判明したHEV陽性血液製剤の輸血を受けた受血者の肝機能およびHEVマーカー(抗HEV抗体、HEV-RNA)を検査した。

結果:ルックバック検査により、HEV陽性血液製剤の輸血を受けた受血者13名が判明した。輸血前検体がHEV RNAまたはHEV抗体陽性だった者はいなかった。HEV感染の兆候を示した4名の受血者のうち、3名がE型肝炎を発症し、1名は一過性のALT上昇(ピーク:611IU/mL)を示した。4本の輸血済み血液製剤におけるHEVウイルス量とgenotypeは、5.4 (G4), 5.5 (G3), 5.8 (G4), 6.8 (G3) log/bagだった。HEV感染を起こさなかった受血者4名に輸血された4製剤では、<4.4 (G3)、<4.4 (G3)、4.3 (G4)、5.5 (G3) log/bagであった。5名は輸血直後に亡くなり、評価できなかった。

結論:血液製剤中のHEV高値(>5.4log/bag)がウイルス伝播に関連付けられると思われた。また、遺伝子型4は遺伝子型3より毒性が高い可能性がある。

使用上の注意記載状況・その他参考事項等

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

血液を原料とすることに由来する感染症伝播等

報告企業の意見

HEV陽性血液の輸血を受けた受血者のルックバック調査から、血液製剤中のHEV高値(>5.4log/bag)がウイルス伝播に関連付けられると考えられたとの報告である。

本剤の製造工程にはコーン分画および液状加熱の2つのウイルス除去・不活化工程が含まれている。最近ある遺伝子型のHEVは耐熱性であるとの成績が発表され、液状加熱の有効性に一部疑念を生じている。しかし、血漿分画製剤で最も長い歴史を持つアルブミンにはHEVの侵淫度が遥かに高い過去から現在に至るまで世界的にHEV感染例がないとの疫学的事実があることから、本剤の安全性は確保されていると考える。

今後の対応

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。

なお、日本赤十字社では、北海道における輸血後HEV感染報告を受け、献血者の疫学調査や、北海道で研究的NATを実施している。

MedDRA/J Ver.12.0J

Monday: Parallel Session S2: TTI

2A-S02-01

PREVALENCE OF HEV INFECTION AMONG JAPANESE BLOOD DONORS

Ikeda H[1], Matsubayashi K[1], Sakata H[1], Takeda H[1], Sato S[1], Kato T[1], Abe I[2], Hino S[3], Tadokoro K[3]

[1]Japanese Red Cross Hokkaido Blood Center, Sapporo, Japan [2]Japanese Red Cross Plasma Fractionation Center, Chitose, Japan [3]Japanese Red Cross Society, Blood Service Headquarters, Tokyo, Japan

Hepatitis E virus (HEV) infection had been recognized to be extremely rare in industrialized countries. Recently, however, increasing reports of hepatitis E including transfusion-transmitted cases are reported in Japan and other industrialized countries.

So far, we have experienced four cases of transfusion-transmitted hepatitis E (TTHE). In 2004, we reported the first molecularly confirmed case of TTHEV, where infection route of the causative donor was not very clear. Then surveys of HEV prevalence in blood donors were undertaken and showed that higher prevalence of IgG anti-HEV in eastern Japan, and the positive rates in female donors were lower than that in male donors. There is a clear age-dependency in IgG anti-HEV prevalence in blood donors in Japan.

Meanwhile, we experienced the second case of TTHE. The causative donor had a barbecue party at a restaurant with his family and enjoyed yakiniku dinner including pig liver and/or intestines. Six of whom including the causative donor and his father were positive for IgM anti-HEV. The father died of fulminant hepatitis E after the barbecue party. HEV isolates from the donor also showed 99.9% homology with that from his father based on nearly entire HEV genome and was classified into genotype 4 that was indigenous to Hokkaido.

This case suggest that there are HEV carriers among blood donors in Japan and HEV infection does not necessarily lead to hepatitis symptoms. For this possibility, we decided to implement preliminary HEV NAT screening in Hokkaido in 2005. However, after the start of this preliminary NAT, we experienced two additional cases of TTHE in 2005 and 2006 because of the delay of the results of NAT. It took almost a week to obtain the results and some blood products, especially platelets concentrates, were already transfused before the NAT. In 2006, we implemented real-time HEV NAT screening.

Up to the end of 2008, the frequency of HEV RNA-positive donors is approximately 1/7700. Male positive donors were dominant. Also, genotype 3 was a dominant genotype. About half of the donors showed the elevation of their ALT level above 45 IU/l during follow-up period. In all of the HEV RNA-positive donors, ALT level came down below 45 IU/l within 50 days after their donation. The HEV RNA-positive donors were also followed-up for their HEV RNA. In all of them, HEV RNA became under the detectable level up to 62-76 days after the donation. The significant increase of the virus level after the donation was observed in 43% of the donors. Compared to hepatitis E patients, a) HEV NAT-positive donors were younger; b) genotype 3 is dominant in contrast to genotype 4 dominance in hepatitis patients. Sequence analyses showed that most of the representative strains from HEV NAT-positive blood donors exhibit over 99% sequence homology with the corresponding swine isolates suggesting that most of HEV are from pigs through food-borne routes.

2A-S02-02

EPIDEMIOLOGY OF HEV INFECTION AMONG BLOOD DONORS IN HOKKAIDO, JAPAN

Matsubayashi K[1], Sakata H[1], Sato S[1], Kato T[1], Abe I[2], Hino S[3], Ikeda H[1]

[1]Japanese Red Cross Hokkaido Blood Center, Sapporo, Japan [2]Japanese Red Cross Plasma Fractionation Center, Chitose, Japan [3]Japanese Red Cross Society, Tokyo, Japan

Background: Recent studies have revealed that indigenous hepatitis E virus (HEV) strains cause domestic hepatitis E in industrialized countries including Japan. Several cases of transfusion-transmitted hepatitis E have been reported there.

Aims: To clarify the characteristics of HEV infection among blood donors in Hokkaido, Japan, and to consider preventive measures for HEV transmission via blood transfusion.

Methods: A total of 1,098,989 serum or plasma samples from blood donors in Hokkaido from January 2005 to December 2008 were tested for the presence of HEV RNA by real-time reverse transcription (RT)-PCR using 20-pooled samples. Blood samples positive for HEV RNA were tested for the presence of IgM and IgG anti-HEV by ELISA, and measured for HEV viral load by real-time RT-PCR. HEV strains from the HEV positive donors were phylogenetically analyzed by direct sequencing of RT-PCR products of regions of HEV ORF1 and ORF2. Questionnaire was mailed to the HEV RNA positive donors to collect the data on their history of intake of animal meats within 2 months previous to the donation. The donors positive for HEV RNA were looked-back and followed-up before and after their positive donations.

Results: HEV RNA was detected in 142 (105 males and 37 females) donors and the overall prevalence of the HEV infected donors was 0.013% [0.015% in males and 0.009% in females] between 2005 and 2008 in Hokkaido. The yearly prevalence of HEV RNA-positives in male and female donors were 0.018% and 0.011% in 2005, 0.016% and 0.013% in 2006, 0.017% and 0.003% in 2007, and 0.012% and 0.009% in 2008, respectively, suggesting progressive expansion of HEV infection in male donors. No clear seasonality of the infection was observed during the period. Of the 142 donors, 109 (77%) donors had neither IgM nor IgG antibodies against HEV at their HEV RNA-positive donations. The strains detected in the donors were segregated into genotype 3 (132) and genotype 4 (6), which were assumed to be Japan-indigenous strains. Of the 103 donors responding to the questionnaire, 71 (69%) had a history of eating the animal viscera such as intestine and/or liver. Of the 39 donors followed-up at least twice a month after the donation, 21 (54%) showed transient elevations of ALT higher than 45 IU/L.

Conclusions: A total of 142 sporadic HEV infection were observed among blood donors during 2005 through 2008 in Hokkaido with male superiority in the prevalence, which were caused by Japan-indigenous HEV strains and appeared to be associated to ingestion of the animal viscera. HEV NAT screening may be more adequate to exclude the HEV-infected donors than HEV antibody screening.

2A-S02-03

LOOK-BACK STUDY ON RECIPIENTS WHO WERE TRANSFUSED HEPATITIS E VIRUS (HEV)-POSITIVE BLOOD

Sato S, Matsubayashi K, Sakata H, Takeda H, Kato T, Ikeda H

Japanese Red Cross Hokkaido Blood Center, Sapporo, Japan

Objective: Up to 2004, we observed at least two cases of transfusion-transmitted HEV infection. Since then, we have implemented NAT screening for HEV in addition to HBV/HCV/HIV-1 in Hokkaido area. The purpose of this study is to evaluate the factor(s) that may lead to transfusion-transmission of HEV by looking back the recipients who were transfused with HEV-positive blood.

Materials and methods: From 2002 to 2004, donor samples with high ALT (>200 IU/mL) were tested for HEV-RNA. From 2005.1-2006.3, all donor samples were screened by HEV-NAT. However, a part of blood products were already transfused before the NAT results turned out. Since 2006.4, blood products have been issued after HEV-NAT screening. The recipients of HEV-positive blood products that were disclosed mostly by look-back

study with stored samples at previous donations were tested for HEV markers including antibody to HEV and HEV-RNA as well as liver functions.

Results: Look-back study disclosed 13 recipients who were transfused HEV-positive blood products. None of them was positive for HEV RNA or anti-HEV in pretransfusion samples. Of four recipients showing signs of HEV infection, three developed hepatitis E and one showed a transient elevation of ALT (peak: 61 IU/mL). The amount and genotypes of HEV in the four transfused blood products were 5.4 [G4], 5.5 [G3], 5.8 [G4] and 6.8 [G3] log/bags, while four blood products that did not cause HEV infection in four recipients contained <4.4 [G3], <4.4 [G3], 4.3 [G4] and 5.5 [G3] log/bags. Five of the 13 recipients died soon after transfusion and were not able to be evaluated for HEV transmission.

Conclusion: The higher amount of HEV (>5.4 log/bag) in blood products may be associated with the virus transmission. Also genotype 4 may be more virulent than genotype 3.

2A-S02-04

ESTABLISHMENT OF A KOREAN HBSAG LOW TITER PERFORMANCE PANEL FOR QUALITY CONTROL OF HBV DIAGNOSTIC KITS

Kwon SY[1], Cho YJ[1], Youn KW[1], Choi KY[1], Joo HA[1], Oh DJ[1], Hwang MW[2], Lee JR[3], Ryu SW[4], Ha GW[5]

[1]Blood Transfusion Research Institute, Korean Red Cross, Seoul, South Korea [2]Blood Services Bureau, Korean Red Cross, Seoul, South Korea [3]The Republic of Korea National Red Cross Plasma Fractionation Center, Eumseong, South Korea [4]Kangwon National University Hospital, Chuncheon, South Korea [5]Animal Genetics, Inc., Suwon, South Korea

Background: Currently, International Standards or commercially available reference materials are used for the validation or quality assessment of domestic *in-vitro* diagnostic medical devices. However, due to their high cost and limited quantity a sustainable supply cannot be guaranteed. Also, these materials might not reflect the viral characteristics common in Korea. This study was conducted to establish a low titer performance panel to be used for quality control of HBV diagnostic kits.

Materials and methods: 371 plasma units with OD values less than 1.0 on EIA screening and 105 units with S/C ratio less than 10.0 on CIA were collected from Korean Red Cross blood centers. HBsAg testing with three EIA assays [GENEDIA HBsAg EIA 3.0 (Green Cross MS), BIO-RAD Monolisa HBsAg Ultra (BIO-RAD), and Murex HBsAg V.3 (Murex Biotec.)] and one CIA assay [Architect HBsAg (Abbott)] was performed on all units. Units with reactive results on CIA or units that were reactive on more than two assays were further subjected to HBV DNA quantification. HBV genotyping and subtyping. Units reactive on HBV DNA quantification were confirmed for HBsAg by HBsAg neutralization. The reactivity of a commercial low titer performance panel to various HBsAg assays was determined to be used as a selection criterion for candidate materials. Based on these results, 13 HBsAg positive units and two HBsAg negative units were selected as candidates. After addition of Bromidox as a preservative, the candidate materials were distributed into the final containers. Collaborative study with seven participating laboratories was conducted using two CIA assays [Architect HBsAg, Prism HBsAg (Abbott)], one ECA assay [Elecsys HBsAg (Roche Diagnostics)], one MEIA assay [AxSYM HBsAg V.2 (Abbott)], and three EIA assays [Retrieval Enzygnost HBsAg 5.0 (Dade Behring), BIO-RAD Monolisa HBsAg Ultra, Murex HBsAg V.3].

Results: Based on the results of the collaborative study, 11 HBsAg positive units and two HBsAg negative units were selected to constitute the low titer performance panel. The mean S/C ratio of HBsAg positive units was less than 10.0 and mean concentration of HBsAg of ten HBsAg positive units was less than 1.0 IU/mL. The panel members were of genotype C, subtype adr and ayr.

Conclusions: As a result of this study, a low titer HBsAg performance panel for quality control of HBV diagnostics kits has been established. This will enable supply of quality control materials at an affordable cost on a long-term basis.

 Vox Sanguinis (2009) **97** (Suppl. 1), 1-179

This research was supported by a grant [08122KFDA274] from Korea Food & Drug Administration in 2008.

2A-S02-05

STATUS OF HEPATITIS VIRAL MARKERS CALCULATED FROM PRETRANSFUSION VIRAL MARKER TEST RESULTS OF PATIENTS AT ASAHIKAWA MEDICAL COLLEGE HOSPITAL

Kino S

Asahikawa Medical College Hospital, Asahikawa, Japan

Background: In October 2004, the Japanese government recommended that six viral markers be tested in patients scheduled for transfusion: hepatitis B surface antigen (HBsAg), antibody to HBsAg (HBsAb), antibody to HBV core antigen (HBcAb), antibody to hepatitis C virus (HCVAb), HCV core antigen (HCVcAg), and antibody to human immunodeficiency virus (HIVAb). At our hospital, we started testing these markers of pretransfusion patients in July 2005.

Aim: Japan is regarded as an endemic area of HBV and HCV. Therefore, it is considered that many Japanese are in a state of asymptomatic or latent HBV or HCV infection. At our hospital, a series of hepatitis marker tests (HBsAg, HBsAb, HBcAb, HCVAb, HCVcAg) was prepared. Physicians used this set menu to evaluate the status of hepatitis viral markers before transfusion. For this study, we calculated the status of hepatitis viral markers at our hospital from results of the pretransfusion viral marker tests conducted routinely before transfusion.

Materials and methods: Hepatitis viral markers of 3353 patients during July 2005 and December 2008 were evaluated. Data were collected from the database of our hospital information system. Measurement methods and positive values were the following: HBsAg (CLIA, >0.5 IU/ml); HBsAb (CLIA, >10 mIU/ml), HBcAb (CLEIA, >70%[NH]), HCVAb (CLIA, >1.0 C.O.I), HCVcAg (CLEIA, >50 fmol/l).

Results: The cases were those of 1721 men and 1632 women. Their average age was 59.9 years (0-96 yrl). The positive rates of HBsAg, HBsAb, HBcAb, HCVAb, and HCVcAg are presented as a table. The rate of positive HBsAb with negative HBcAb was 8.9%, the rate of negative HBsAb with positive HBcAb was 9.9%, the rate of both positive was 20.3%, and the rate of both negative was 60.9%. Among 204 HCVAb positive cases, 118 cases were HCVcAg positive. The others were HCVcAg negative. No HCVcAg positive case was HCVAb negative. Among the 107 cases that were positive for both some HBV marker and some HCV marker, 88 cases were HBcAb positive.

Summary: We determined the status of hepatitis viral markers of a hospital based on results of pretransfusion viral tests. We assessed the status of nationwide or latent hepatitis viral infection from a hospital level to a nationwide level if a pretransfusion viral marker test were strictly implemented for all patients scheduled for transfusion. Furthermore, these data provide background information for developing preventive measures against hepatitis viral infections, including transfusion-transmitted infections and hospital infections.

Table 1. Age related positive rate of viral marker

age	number of pts.	positive rate of viral marker				
		HBsAg	HBsAb	HBcAb	HCVAb	HCVcAg
0-9 yr	87	1.1%	6.9%	3.4%	0.0%	0.0%
10-19 yr	42	0.0%	2.4%	0.0%	2.4%	0.0%
20-29 yr	130	1.5%	12.3%	4.6%	1.6%	0.0%
30-39 yr	266	2.3%	14.7%	6.6%	1.5%	0.4%
40-49 yr	285	3.2%	26.0%	15.4%	3.5%	1.1%
50-59 yr	535	6.5%	30.1%	32.5%	4.3%	3.1%
60-69 yr	755	7.3%	38.7%	41.3%	8.5%	4.7%
70-79 yr	900	1.6%	32.2%	34.4%	9.1%	6.1%
80-89 yr	323	1.2%	38.4%	40.9%	7.4%	3.1%
over 90 yr	27	0.0%	25.9%	29.6%	0.0%	0.0%
total	3353	3.7%	29.2%	30.2%	6.3%	3.6%

別紙様式第2-1

No. 17

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
				2009. 12. 20	該当なし	
一般的名称	人血清アルブミン		研究報告の公表状況	Iwanaga M, Koga Y, Soda M, Inokuchi N, Sasaki D, Hasegawa H, Yanagihara K, Yamaguchi K, Kamihira S, Yamada Y. 51st ASH Annual Meeting and Exposition; 2009 Dec 5-8; New Orleans.	公表国 日本	
販売名(企業名)	赤十字アルブミン20(日本赤十字社) 赤十字アルブミン25(日本赤十字社) 赤十字アルブミン20%静注4g/20mL(日本赤十字社) 赤十字アルブミン20%静注10g/50mL(日本赤十字社) 赤十字アルブミン25%静注12.5g/50mL(日本赤十字社)					

研究報告の概要

○ヒトT細胞白血病ウイルス1型(HTLV-1)有病率の傾向および長崎(日本)の成人T細胞白血病/リンパ腫(ATL)の発症率:病院ベースおよび集団ベース試験

序論:HTLV-1の有病率は、主に献血者の年齢別抗体陽性率により評価されATL発症率が推定されてきたが、献血者集団の特性から過小評価されている可能性がある。献血者以外のHTLV-1キャリアの出生年別ATL発症率データは少ない。

方法:2000～2007年に長崎大学病院を受診した患者10,261名(男性:5,523、女性:4,737)のHTLV-1抗体検査のデータ、及び長崎県がん登録中の長崎市で診断されたATL症例360例(男性:188、女性:172)のデータを評価した。長崎市の2006国勢調査人口に病院ベースの陽性率データを適用して、HTLV-1キャリアの出生年別ATL発症率を推定した。

結果:患者10,261名のうち、HTLV-1抗体陽性者は1,392名(男性:653、女性:739)、陽性率は13.57%(95%CI:12.90-14.23%)であった。陽性率は女性が有意に高かった(15.60%対11.82%、P<0.0001)。出生年別抗体陽性率は、18.69%(1926年以前)、17.83%(1927-1936)、15.91%(1937-1946)、13.80%(1947-1956)、9.19%(1957-1966)、4.07%(1967-1976)、2.07%(1977-1986)、0%(1987年以降)であった(有意な減少傾向:P < 0.0001)。長崎市の出生年別の年間HTLV-1キャリア推定人数は、それぞれ5257、8093、8151、8083、4434、2180、785、0であった。キャリア100,000人あたりの年間ATLL発症率の推定は、それぞれ171、86、41、32、11、0、0、0となった。HTLV-1キャリアの生涯の粗ATL発症リスクは、男性7.29%、女性3.78%と推定された。

結論:本試験の出生年別HTLV-1抗体陽性率は供血者の陽性率より約50%高く、流行地域で高齢者の大規模なキャリア集団が現在も存在することを示唆している。発症予防のためATL発現機序を解明するには更なる試験が必要である。

使用上の注意記載状況・その他参考事項等

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

血液を原料とすることに由来する感染症伝播等

報告企業の意見	今後の対応
長崎大学病院を受診した患者の出生年別HTLV-1抗体陽性率は過去に報告された供血者の陽性率と比べて約50%高く、流行地域において高齢者のHTLV-1キャリアの大規模集団が存在することが示唆されたとの報告である。 HTLV-1は脂質膜を有するRNAウイルスである。垂直感染により日本では古代から広く侵淫しているが、本製剤による感染の報告はない。本製剤の製造工程には、平成11年8月30日付医薬発第1047号に沿ったウイルス・プロセスバリデーションによって検証された2つの異なるウイルス除去・不活化工程が含まれているとから、本製剤の安全性は確保されていると考える。	日本赤十字社では献血時のスクリーニング法として、より感度の高い化学発光酵素免疫測定法(CLEIA)によるHTLV-1抗体のスクリーニング検査を行っている。今後も引き続き情報の収集に努める。

Blood (ASH Annual Meeting Abstracts) 2009 114: Abstract 1920

Poster Session

NON-HODGKIN'S LYMPHOMA - BIOLOGY, EXCLUDING THERAPY POSTER I

Trends in Human T-Cell Leukemia Virus Type-1 (HTLV-1) Prevalence and the Incidence of Adult T-Cell Leukemia/Lymphoma (ATL) in Nagasaki, Japan: A Hospital-Based and Population-Based Study.

Masako Iwanaga[1], Yoshito Koga[*,2], Midori Soda[*,3], Naoko Inokuchi[*,2], Daisuke Sasaki[*,4], Hiroo Hasegawa[*,5], Katsunori Yanagihara[*,4], Kazunari Yamaguchi[*,6], Shimeru Kamihira[*,5] and Yasuaki Yamada[5]

[1] Hematology and Molecular Medicine, Nagasaki University, Nagasaki, Japan,
[2] Nagasaki University Graduate School of Biomedical Sciences,
[3] Radiation Effects Research Foundation,
[4] Laboratory Medicine, Nagasaki University Graduate School of Biomedical Sciences, Nagasaki, Japan,
[5] Nagasaki University Graduate School of Biomedical Sciences, Nagasaki, Japan,
[6] Safety Research on Blood and Biological products, National Institute of Infectious Diseases, Musashimurayama-shi, Japan

Abstract 1920

Poster Board I-943

Introduction: The prevalence of HTLV-1 is mostly evaluated by the age-specific seroprevalence in blood donors, and the results have been conventionally used to estimate the age-specific incidence of ATL in Japan. However, the results may be underestimated due to an age limit (16-69 yr) for donation, a healthy donor effect, and a birth cohort effect. Data concerning the birth-year specific incidence of ATL among HTLV-1 carriers other than blood donors are scarce.

Methods: The study evaluated data of the anti-HTLV-1 antibody testing of 10,261 patients (males: 5,523, females: 4,737) who visited the Nagasaki University Hospital during 2000-2007 and data of 360 ATL cases (males: 188, females: 172) who were diagnosed in Nagasaki City (an endemic area in Japan) in a population-based Nagasaki Prefectural Cancer Registry (NPCR). To estimate birth-year specific incidence rates of ATL in population-based HTLV-1 carriers, we used the 2006 census population for Nagasaki City by applying the hospital-based seroprevalence data.

Results: Of 10,261 patients, 1,392 (males: 653, females: 739) were HTLV-1 antibody positive. The overall HTLV-1 seroprevalence was 13.57% (95%CI: 12.90-14.23%). The seroprevalence was significantly higher in females than in males (15.60% vs. 11.82%, P<0.0001). The birth-year specific seroprevalence was 18.69% (before 1926), 17.83% (1927-1936), 15.91% (1937-1946), 13.80% (1947-1956), 9.19% (1957-1966), 4.07% (1967-1976), 2.07% (1977-1986), and 0% (after 1987) (a significantly declining trend: P <0.0001). The estimated annual number of HTLV-1 carriers by birth-year in Nagasaki city was 5257, 8093, 8151, 8083, 4434, 2180, 785, and 0, respectively. Finally, we estimated the annual incidence rate of ATLL per 100,000 HTLV-1 carriers by birth-year; 171 (before 1926), 86 (1927-1936), 41 (1937-1946), 32 (1947-1956), 11 (1957-1966), and 0 (after 1967). The crude lifetime risk of developing ATLL in HTLV-1 carriers was estimated to be 7.29% for males and 3.78% for females.

Conclusions: The birth-year specific HTLV-1 seroprevalnces in the present study were approximately 50% higher than those previously reported in blood donors1 (for example: 6.22% in those born before 1950). Although it is possible that our results are over-estimated2, the present study suggests that there is still a large pool of elderly HTLV-1 carriers in this endemic area. Further studies are needed to investigate the mechanism of the development of ATL among HTLV-1 carriers for preventing the development. Reference: 1) Iwanaga M et al. Int J Hematol, 2009. 2) Arisawa K et al. Int J Cancer, 2000.

Disclosures: No relevant conflicts of interest to declare.

Footnotes

* Asterisk with author names denotes non-ASH members.

別紙様式第2-1

No. 16

医薬品 研究報告 調査報告書

識別番号・報告回数			報告日	第一報入手日	新医薬品等の区分	総合機構処理欄
				2009. 12. 20	該当なし	
一般的名称	人血清アルブミン		研究報告の公表状況	Sobata R, Matsumoto C, Suzuki K, Uchida S, Suzuki Y, Satake M, Tadokoro K. XIVth Regional Congress of the ISBT, Asia; Nov 14-18, 2009; Nagoya.	公表国 日本	
販売名(企業名)	赤十字アルブミン20(日本赤十字社) 赤十字アルブミン25(日本赤十字社) 赤十字アルブミン20%静注4g/20mL(日本赤十字社) 赤十字アルブミン20%静注10g/50mL(日本赤十字社) 赤十字アルブミン25%静注12.5g/50mL(日本赤十字社)					

研究報告の概要

○ヒトT細胞リンパ球向性ウイルス1型(HTLV-1)抗体陽性献血者のプロウイルス量の定量と輸血感染症の感染性ウイルス量の評価

背景:献血血液の抗体スクリーニングと保存前白血球除去は、日本におけるHTLV-1の輸血感染(TTI)リスクを確実に低下させた。しかし、TTIを生じる血液成分中プロウイルス量は不明である。

目的:HTLV-1抗体陽性献血者から白血球除去前に採取した血液検体のプロウイルス量を調べた。献血者のプロウイルス量の分布と過去のデータから、感染性を持つプロウイルス量を推定した。

方法:献血者74名の保管検体の末梢血単核細胞または凝固血液からDNAサンプルを得た。検体は白血球除去前に採取した。HTLV-1pX領域とヒトCD81遺伝子のTaqMan PCRでプロウイルス量を測定し、細胞DNA量を推定した。過去のデータでは、抗体陽性献血者由来の赤血球濃厚液1単位の輸血を受けた患者約80%にセロコンバージョンが起こったことから、血液検体の80%に感染リスクがあると仮定し、輸血感染力を持つウイルス量を推定した。

結果:献血者のHTLV-1プロウイルス量は、100白血球につき、0.01～4.9コピー(平均0.83)だった。評価した検体の80%には、100白血球あたり少なくとも0.06コピーのプロウイルスが含まれていた。赤血球濃厚液1単位あたりの白血球数を白血球除去前は1×10^9と仮定すると、TTIを生じた製剤には最低6×10^5のHTLV-1感染細胞が含まれる。

結論:2007年、日本のすべての血液製剤に保存前白血球除去が導入され、残存白血球数は製剤の99%で1×10^6未満と確認されている。スクリーニング未実施でもHTLV-1感染細胞の最大数は1製剤につき4.9×10^4と考えられ、推定される感染性ウイルス量(6×10^5の感染細胞)よりかなり低い。抗体スクリーニングと白血球除去は、日本におけるHTLV-1のTTIリスクを事実上排除した。

使用上の注意記載状況・その他参考事項等

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

血液を原料とすることに由来する感染症伝播等

報告企業の意見	今後の対応
HTLV-1抗体陽性供血者の血液成分中のHTLV-1感染細胞の最大数は推定される感染性ウイルス量よりかなり低く、血清学的スクリーニングと白血球除去によって輸血感染リスクは事実上排除されているとの報告である。 HTLV-1は脂質膜を有するRNAウイルスである。垂直感染により日本では古代から広く侵淫しているが、本製剤による感染の報告はない。本製剤の製造工程には、平成11年8月30日付医薬発第1047号に沿ったウイルス・プロセスバリデーションによって検証された2つの異なるウイルス除去・不活化工程が含まれているとから、本製剤の安全性は確保されていると考える。	日本赤十字社では献血時のスクリーニング法として、より感度の高い化学発光酵素免疫測定法(CLEIA)によるHTLV-1抗体のスクリーニング検査を行っている。今後も引き続き情報の収集に努める。

MedDRA/J Ver.12.0J

JRC2009T-068

P-141

QUANTIFICATION OF HUMAN T-CELL LYMPHOTROPIC VIRUS TYPE 1 (HTLV-1) PROVIRUS LOAD IN SEROPOSITIVE BLOOD DONORS AND ESTIMATION OF INFECTIOUS VIRAL LOAD FOR TRANSFUSION-TRANSMITTED INFECTION

Sobata R[1], Matsumoto C[1], Suzuki K[1], Uchida S[1], Suzuki Y[2], Satake M[1], Tadokoro K[1]

[1]*The Japanese Red Cross Central Blood Institute, Tokyo, Japan* [2]*The Japanese Red Cross Tokyo Metropolitan Blood Center, Tokyo, Japan*

Background: Serological screening and prestorage leukocyte reduction for donated blood have undoubtedly decreased the risk of transfusion-transmitted infection (TTI) for HTLV-1 in Japan. However, the provirus load in blood component that would cause TTI is still unclear.

Aims: HTLV-1 provirus load was measured in blood samples collected before leukocyte reduction that were obtained from seropositive blood donors. From the distribution of provirus load among blood donors, provirus load for infectivity was estimated using the historical data on the frequency of transfusion-transmitted infection.

Methods: DNA samples were obtained from peripheral blood mononuclear cells or blood clots of stored samples obtained from 74 HTLV-1-seropositive individuals. All blood samples were obtained before leukocyte reduction. HTLV-1 provirus load was determined using TaqMan PCR for the HTLV-1 pX region and human CD81 gene to estimate the amount of cellular DNA. Previous data showed that seroconversion occurred in approximately 80% of patients transfused with one unit of fresh red-cell concentrate from HTLV-1-seropositive blood donors (Okochi K et al. ADS Res 1986;2:157–61). It is, therefore, expected that 80% of our blood samples will be in the category of units with infectious risk, which allows us to estimate the viral load for infectivity by transfusion.

Results: The HTLV-1 provirus loads in HTLV-1-seropositive blood donors ranged from less than 0.01 to 4.9 copies (average 0.83) per 100 leukocytes. Eighty per cent of blood samples evaluated contained at least 0.06 copies of HTLV-1 provirus per 100 leukocytes. Assuming that the number of leukocytes per unit of red-cell concentrate was $1\text{-}10^9$ before leukocyte reduction, a minimum of $6\text{-}10^5$ HTLV-1-infected cells would have been found in the unit that caused TTI.

Conclusions: In 2007, universal prestorage leukocyte reduction was introduced for all blood components in Japan. The number of residual leukocytes after leukocyte reduction is confirmed to be less than $1\text{-}10^6$ in 99% of unit currently issued from Japanese Red Cross Blood Center. If serological screening is omitted, the maximum number of HTLV-1-infected cells found in blood components would be $4.9\text{-}10^4$ per unit. This figure is substantially lower than the infectious virus load estimated ($6\text{-}10^5$ infected cells). The combination of serological screening and universal leukocyte reduction virtually eliminated the TTI risk for HTLV-1 in Japan.

Journal compilation © 2009 International Society of Blood Transfusion *Vox Sanguinis* (2009) 97 (Suppl. 1), 1–179

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
				2009. 11. 19	該当なし	
一般的名称	新鮮凍結人血漿				公表国	
販売名(企業名)	新鮮凍結血漿「日赤」(日本赤十字社) 新鮮凍結血漿-LR「日赤」(日本赤十字社) 新鮮凍結血漿-LR「日赤」成分採血(日本赤十字社)		研究報告の公表状況	Agapova M, Custer B. AABB Annual Meeting and TXPO; 2009 Oct. 24-27; New Orleans.	米国	

研究報告の概要

○米国の血液供給における*T. cruzi*スクリーニングの費用対効果

背景:シャーガス病の病原体である*Trypanosoma cruzi* (*T. cruzi*)は、輸血の安全性を脅かしている。現在、米国の供血血液の75～80%に*T. cruzi*のスクリーニング検査が行われており、29,000名当たり1名が陽性と考えられる。病原体の輸血による感染性とシャーガス病の拡大については特徴が十分に分かっておらず、全国的なスクリーニングの費用対効果は報告されていない。

方法:*T. cruzi*の脅威とその制圧にかかる費用を評価するために、疾患の進行モデルを用いて受血者の仮想集団の生涯コストと健康アウトカムを異なるスクリーニング計画下で比較した。以下の7つの供血者・供血血液検査方法を分析し、スクリーニング非実施と比較した:1)ラテンアメリカで生まれた供血者、2)血小板供血、3)初回供血者、4)全供血者を1回、5)全供血者を2回、6)全血と血小板供血、7)すべての供血。モデルパラメータは、スクリーニング・データまたは文献レビューより得た。一元感度分析および確率的感度分析を用いて、影響力のあるパラメータと全体の不確実性を評価した。

結果:各方法の費用対効果は、1)170,000、2)330,000、3)370,000、4)760,000、5)970,000、6)1,070,000、7)1,360,000(単位:ドル/QALY)となった。モデルにおける最も影響力のあるパラメータは、受血者の特徴と関連がある(生存率、健康状態ユーティリティ、将来の健康状態の低下率)。最も影響が強かったのは*T. cruzi*の血清陽性率と伝播効率だった。CE比率は、米国が報告した血清陽性率1/3,333と1/100,000の範囲ではそれぞれ、92%低く(よい)、215%高かった(悪い)。このモデルは、シャーガス病に伴う変数に無関係だった。

結論:本分析では、選択的*T. cruzi*スクリーニング検査は、全数検査とほぼ同等の効果があり、コストが低いことを示している。これらの所見は、輸血による伝播が観察されなかった、2年間の試験およびルックバック・データと整合している。

使用上の注意記載状況・その他参考事項等

新鮮凍結血漿「日赤」
新鮮凍結血漿-LR「日赤」
新鮮凍結血漿-LR「日赤」成分採血

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

米国の血液供給における*T. cruzi*検査において、選択的スクリーニングは全数検査とほぼ同等の効果があり、コストが低いことが示されたとの報告である。

今後の対応

日本赤十字社は、輸血感染症対策として献血時に海外渡航歴の有無を確認し、帰国(入国)後4週間は献血不適としている。また、シャーガス病の既往がある場合には献血不適としている。日本在住の中南米出身献血者については、厚生労働科学研究「献血血の安全性確保と安定供給のための新興感染症等に対する検査スクリーニング法等の開発と献血制限に関する研究」班と共同して検討する予定である。今後も引き続き情報の収集に努める。

4

MedDRA/J Ver.12.1J

TRANSFUSION
2009-Vol. 49 Supplement

time. Finally, for donors with previously negative donations and recent travel to Latin America, there was no evidence of incident infection. **Conclusion:** While country of birth is the best predictor of *T. cruzi* infection, these data indicate that selective testing based solely on donor responses to any question or combination of questions would be insufficient to identify all RIPA+ *T. cruzi* infections. These data also have helped in guiding the development of a large incidence study to accompany conversion to selective 1-time testing of all donors in most of the US.

Disclosure of Commercial Conflict of Interest

M. Agapova: Nothing to disclose; H. H. Biswas: Nothing to disclose; M. P. Busch: Nothing to disclose; B. Custer: Nothing to disclose; H. Kamel: Nothing to disclose; P. Tomasulo: Nothing to disclose

Disclosure of Grants Conflict of Interest

M. Agapova: Nothing to disclose; H. H. Biswas: Nothing to disclose; M. P. Busch: Gen-Probe, Inc., Grants or Research Support, Travel Support or Honorarium Chiron / Novartis, Grants or Research Support, Travel Support or Honorarium Ortho, Travel Support or Honorarium Abbott, Travel Support or Honorarium; B. Custer: Nothing to disclose; H. Kamel: Nothing to disclose; P. Tomasulo: Nothing to disclose

Birth Country or Region	Allogeneic Donors Number (%) N = 703,725	*T.cruzi* RIPA+ Number (%) N = 34	*T.cruzi* RIPA+ Prevalence by Birth Country or Region
USA	546,230 (77.2)	10 (29.4)	1:54,623
Mexico	18,511 (2.6)	10 (29.4)	1:1,851
Central or South America	2,235 (0.3)	8 (23.5)	1:279
All other countries	13,254 (1.9)	1 (2.9)	1:13,254
Missing/Unreported	123,495 (17.5)	5 (14.7)	1:24,699

S39-020D

Sensitivity of Selective Testing for Antibody to *Trypanosoma cruzi* (*T. cruzi*)

S L Stramer[1] (slstramer@usa.redcross.org), R L Townsend[1], G A Foster[1], D Krysztof[2], D A Leiby[3], J P Brodsky[3], C Rouault[3], B A Lenes[3], R Y Dodd[3]; [1]Scientific Support Office, American Red Cross, Gaithersburg, MD, USA; [2]Holland Laboratory, American Red Cross, Rockville, MD, USA; [3]Quality Analytics, Riverwoods, IL, USA; [4]Community Blood Center of South Florida, Lauderhill, FL, USA

Background: Donor screening for *Trypanosoma cruzi* antibody began in 2007 at the American Red Cross by testing each donation (dn) from every donor (universal testing). Data for the 22-month experience with universal testing (Jan 27 07-Nov 30 08) were examined to determine the sensitivity of selective testing. Donor data collected included: risk factors related to direct or indirect exposure in a non-US endemic country, US-derived infection, ELISA false negativity (neg) and potential incident infection. **Methods:** The Ortho *T. cruzi* ELISA was used to screen each dn. Repeat reactive (RR) dns were further tested using a research radioimmunoprecipitation assay (RIPA); RIPA-pos donors were considered confirmed. RR donors were followed and tested by repeat serologic/parasitologic tests (PCR/ hemoculture-HC). Donors were also asked to respond to a detailed survey regarding risk factors; RIPA-pos donors were defined as cases and RIPA-unconfirmed as controls. **Results:** Prevalence for ~13 million dns screened was 1:36,000 (RR rate = 0.014%) and identified 394 RIPA-pos donors of which 4-14% were pos for the presence of the parasite by PCR/HC. Of 157 pos donors who completed a risk survey, all but 40 were born in an endemic area compared to 5/457 controls ($p < 0.0001$; OR 256 by univariate and 32 by multivariate analyses). The 40 US-derived cases came from 18 states; 6 had congenital infection and 7 others had identified risks (2 due to residence in an endemic area prior to *T. cruzi* screening and 5 with outdoor activities in the Southern US); 16/394 (4%) ELISA RR/RIPA pos donors had prior ELISA false-neg donation results of which 11 had one prior neg dn and 5 had >1 prior neg dn; 8/16 had prior neg reactivity within 20% of the ELISA cutoff. A 20% reduction in the assay cutoff would increase the RR rate by 0.025%. None of the 16 was PCR/HC pos, and of those followed (13/16), all ELISA signals were stable and none represented incident cases. No incident donors were identified in 2.5 million donors with >2 neg dns during the 22 months (>2.3 million person years of observation; neg dn interval = 0.9 years). Sensitivity by method is provided in the Table. **Conclusions:** A selective testing strategy based on qualifying a donor by a single direct or indirect test had high sensitivity. A protocol to further determine *T. cruzi* donor incidence is under development with collaborators from Blood Systems.

Disclosure of Commercial Conflict of Interest

J. P. Brodsky: Abbott Laboratories, Stocks or Bonds; R. Y. Dodd: Nothing to disclose; G. A. Foster: Nothing to disclose; D. Krysztof: Nothing to disclose; D. A. Leiby: Nothing to disclose; B. A. Lenes: Nothing to disclose; C. Rouault: Nothing to disclose; S. L. Stramer: Nothing to disclose; R. L. Townsend: Nothing to disclose

Disclosure of Grants Conflict of Interest

J. P. Brodsky: Nothing to disclose; R. Y. Dodd: Nothing to disclose; G. A. Foster: Nothing to disclose; D. Krysztof: Nothing to disclose; D. A. Leiby: Nothing to disclose; B. A. Lenes: Nothing to disclose; C. Rouault: Nothing to disclose; S. L. Stramer: Nothing to disclose; R. L. Townsend: Nothing to disclose

Method	# Detected/Tested	% Sensitivity (95% CI)
Universal Testing (Ortho)	860/861	99.88 (99.35-100)
Endemic birth question	117/157	74.52 (67.83-81.87)
1 × neg	378/394	95.94 (93.49-97.66)
2 × neg	389/394	98.73 (97.06-99.59)

S39-020D

Cost-Effectiveness of Screening for *T. cruzi* in the US Blood Supply

M Agapova[1] (magapova@bloodsystems.org), B Custer[1], Epidemiology/Outcomes Research, Blood Systems Research Institute, San Francisco, CA, USA

Background: *Trypanosoma cruzi* (*T. cruzi*), the etiologic agent of Chagas disease is a safety threat to transfusion. Currently, 75-80% of US donations are screened for *T. cruzi*. Overall, 1 donor out of 29,000 is expected to be confirmed *T. cruzi* positive. The transmissibility of the pathogen by transfusion and progression to Chagas disease are not well characterized in the US, and the cost-effectiveness of nation-wide screening has not been reported. **Methods:** To evaluate the threat of *T. cruzi* as well as costs associated with intercepting this threat, we used disease progression modeling to compare lifetime costs and health outcomes of a hypothetical cohort of blood recipients under different screening strategies. We analyzed 7 donor or donation testing strategies: 1) donors born in Latin America; 2) platelet donations; 3) first-time donors; 4) all donors one time; 5) all donors from laboratory screening data or literature review. One-way and probabilistic sensitivity analyses were used to assess influential parameters and overall uncertainty. **Results:** Costs, effectiveness and the cost-effectiveness (CE) of each strategy compared to no testing, are provided in the table. The cost-effectiveness of testing all donors one time is $760,000/QALY, all donors two times is $970,000/QALY and universal testing is $1.36MQALY. The most influential parameters in the model are related to characteristics of the transfused population: survival rate, health state utilities and discount rate for future health states. With respect to *T. cruzi* seroprevalence and transmission efficiency are the most influential. CE ratios were 92% lower (better) and 215% higher (worse) between the limits of US-reported seroprevalence, 1/3333 and 1/100,000, respectively. The model was insensitive to variables associated with Chagas disease. **Conclusions:** This analysis suggests that selective *T. cruzi* screening generates nearly the same effectiveness as universal screening, but at reduced cost. These findings are consistent with 2-years of testing and lookback data, where incident infections or substantial transmission by transfusion have not been observed.

Disclosure of Commercial Conflict of Interest

M. Agapova: Nothing to disclose; B. Custer: Nothing to disclose

Disclosure of Grants Conflict of Interest

M. Agapova: Nothing to disclose; B. Custer: Nothing to disclose

Testing Strategy	Cost ($)	Effectiveness (QALYs)	CE Ratio ($/QALYs)	CE Ratio 95% range ($/QALYs)
No testing	0.06	8.56605503	-	-
Born in Latin America	0.48	8.56605609	170,000	55,000-380,000
Platelets	0.81	8.56605772	330,000	120,000-690,000
First time	1.36	8.56605906	370,000	160,000-710,000
One time all	3.54	8.56606013	760,000	350,000-1,410,000
Two time all	4.61	8.56606024	970,000	450,000-1,810,000
Whole Blood/Platelets	5.41	8.56605027	1,070,000	500,000-2,010,000
Universal	6.56	8.56606030	1,360,000	640,000-2,530,000

別紙様式第2-1

No. 9

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
				2009. 11. 5	該当なし	
一般的名称	人血清アルブミン		研究報告の公表状況	Azevedo RSS, Silva EVP, Carvalho VL, Rodrigues SG, Nunes Neto JP, Monteiro HAO, et al. Emerg Infect Dis. 2009 Nov;15(11):1830-2.	公表国 ブラジル	
販売名(企業名)	赤十字アルブミン20(日本赤十字社) 赤十字アルブミン25(日本赤十字社) 赤十字アルブミン20%静注4g/20mL(日本赤十字社) 赤十字アルブミン20%静注10g/50mL(日本赤十字社) 赤十字アルブミン25%静注12.5g/50mL(日本赤十字社)					

研究報告の概要

○マヤロ熱ウイルス、ブラジル・アマゾン

マヤロウイルスはアルファウイルス属トガウイルス科で、ジェノタイプDとLの2系統が確認されている。流行地は南米の熱帯地域で、発疹、発熱、重い関節痛などのデング様の疾患と関連している。関節痛は数週間持続することもある。

2008年2月、マヤロ熱ウイルス(MAYV)のアウトブレイクが、ブラジル北部、パラー州サンタバーバラ県のベレム近郊の村で発生した。村の住民は150名程度で多くは貧しく、密林の真ん中の木製の家に住んでいた。発熱を訴えた105名のうち53名は村の住民、52名は農学専攻の学生で村の近隣の施設に1週間滞在していた。

患者は発疹、発熱、重い関節痛の症状を呈し最長7日間持続した。患者の血清検体のIgMをELISAで検査したところ、36検体からIgMが検出された。MAYV分離株3株がジェノタイプDと確認され、系統発生解析では、1991年にブラジル北部で分離された株と近縁であることが明らかとなった。

また、村で蚊を捕獲したところ、832匹のうち188匹がMAYVの主要な媒介蚊である*Haemagogus janthinomys*だった。蚊から採取された検体及び患者の急性期血清検体がマウスに感染性を持つことが確認された。

使用上の注意記載状況・その他参考事項等

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

血液を原料とすることに由来する感染症伝播等

報告企業の意見	今後の対応
ブラジル北部、パラー州サンタバーバラ県のベレム近郊の村で、マヤロ熱ウイルスの流行が見られたとの報告である。 マヤロ熱ウイルスは脂質膜を持つ中型のRNAウイルスで、これまで本製剤によるマヤロ熱発症の報告はない。本製剤の製造工程には、平成11年8月30日付医薬発第1047号に沿ったウイルス・プロセスバリデーションによって検証された2つの異なるウイルス除去・不活化工程が含まれていることから、本剤の安全性は確保されていると考える。	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(入国)後4週間は献血不適としている。また、発熱などの体調不良者を献血不適としている。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。

28

5

MedDRA/J Ver.12.0J

JRC2009T-059

DISPATCHES

Mayaro Fever Virus, Brazilian Amazon

Raimunda S.S. Azevedo, Eliana V.P. Silva, Valéria L. Carvalho, Sueli G. Rodrigues, Joaquim P. Nunes Neto, Hamilton A.O. Monteiro, Victor S. Peixoto, Jannifer O. Chiang, Márcio R.T. Nunes, and Pedro F.C. Vasconcelos

In February 2008, a Mayaro fever virus (MAYV) outbreak occurred in a settlement in Santa Barbára municipality, northern Brazil. Patients had rash, fever, and severe arthralgia lasting up to 7 days. Immunoglobulin M against MAYV was detected by ELISA in 36 persons; 3 MAYV isolates sequenced were characterized as genotype D.

Mayaro virus (MAYV) is a member of the family *Togaviridae* and the genus *Alphavirus*. Recent molecular studies have recognized 2 MAYV lineages: genotypes D and L (*1*). MAYV has been associated with a dengue-like illness with rash, fever, and severe arthralgia in tropical South America. Arthralgia lasts for several weeks and affects principally ankles, wrists, and toes, but also can affect major joints. MAYV causes a mild to moderately severe acute febrile illness of 3–5 days' duration with uneventful recovery (*2*).

The Study

In February 2008, an outbreak of a dengue-like illness was reported in the Pau D'arco settlement, 38 km from Belém, Para state, in the Brazilian Amazon (online Appendix Figure, available from www.cdc.gov/EID/content/15/11/1830-appF.htm). This rural community has 48 houses with ≈150 inhabitants, many of whom live in poor conditions. They reside in the middle of a native forest, in softwood houses, in the municipality of Santa Barbara (2007 population ≈14,439).

A total of 105 persons were examined in a house-to-house survey. They reported a febrile illness within the past 30 days, had a current febrile illness, or reported contact with persons with febrile illness. Fifty-three resided in the settlement (50 were agricultural workers), and 52 were agronomy students at a public university in Belém and had been training for a week at a field station adjacent to the settlement. The students slept in the station for a week; their activities included periodic visits to the settlement and sporadic ingression to the forest. Students and agricultural workers were bled weekly by convenience from March 17 through April 4, 2008. All serum samples were processed by ELISA for detection of immunoglobulin (Ig) M (*3*).

During the same diurnal period (9:00 AM–3:00 PM), mosquitoes were captured in the settlement by using human bait on the ground and in the forest canopy (≈15 m high) near the residences. A total of 832 (49 lots) *Culicidae* mosquitoes were collected and frozen before being used for virus isolation. Of these, 188 (11 lots) were *Haemagogus janthinomys*, the main vector of MAYV; the remaining 644 (38 lots) were mainly members of the genera *Wyeomyia, Aedes, Sabethes*, and *Limatus*.

Newborn mice (*Mus musculus*) and C6/36 cells were inoculated with acute-phase serum from samples collected from febrile patients and pooled mosquitoes, as previously described (*4*,*5*). The inoculated animals and cells were observed daily, and the presence of virus was confirmed by complement fixation and immunofluorescent assays (*4*). Three MAYV strains were isolated: 2 from febrile persons and 1 from a pool with 2 *H. janthinomys* mosquitoes collected at ground level. All 3 strains were isolated with both assays.

IgM was detected in 36 (34%) serum samples (Figure 1, panel A). Of those 36 samples, 23 (64%) were collected from residents of the settlement, and 13 (36%) were from residents of Belém and Ananindeua municipalities; these persons had visited the settlement area for a week (Figure 2, panel B). Persons with Mayaro fever ranged in age from 4 to 55 years, and 21 (58%) were male (Figure 1, panel C). Fifty-two percent of MAYV-positive persons were students, 31% were agriculturists, and 17% participated in other activities (Figure 1, panel D).

Of the 36 MAYV-infected persons, 33 were symptomatic. Illness was characterized by sudden onset of fever (100% of patients), arthralgia (89%), myalgia (75%), headache (64%), articular edema (58%), rash (49%), and retroocular pain (44%). Other less frequent symptoms were itching (33%), dizziness (25%), anorexia (22%), swollen lymph nodes (17%), and vomiting (4%).

Other common exanthematic illnesses in Brazil included in the differential diagnoses were dengue fever, rubella, B19 parvovirus, human herpesvirus 6, infectious mononucleosis, malaria, and yellow fever. Serologic results excluded these illnesses.

RNA was extracted by using the TRIZOL LS (Invitrogen, Carlsbad, CA, USA) reagent method according to the manufacturer's instructions. Envelope (E)2 and E1 genes of the MAYV genome were amplified by using a standard 1-step reverse transcription–PCR protocol, as pre-

Author affiliations: Instituto Evandro Chagas, Ananindeua, Brazil (R.S.S. Azevedo, E.V.P. Silva, V.L. Carvalho, S.G. Rodrigues, J.P. Nunes Neto, H.A.O. Monteiro, J.O. Chiang, M.R.T. Nunes, P.F.C. Vasconcelos); and Universidade do Estado do Pará, Belém, Brazil (V.S. Peixoto, P.F.C. Vasconcelos)

DOI: 10.3201/eid1511.090461

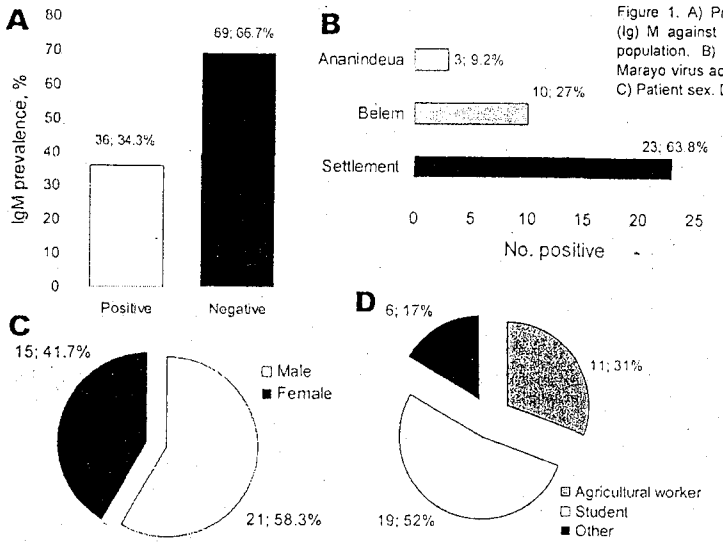


Figure 1. A) Prevalence of immunoglobulin (Ig) M against Mayaro virus in the studied population. B) Prevalence of IgM against Marayo virus according to area of residence. C) Patient sex. D) Patient work activities.

viously described (*1*). The cDNA products were directly sequenced (*6*).

We conducted phylogenetic analysis by using the maximum parsimony (heuristic algorithm), neighbor-joining (Kimura 3-parameter and F84 corrections), and maximum-likelihood methods (*7*) implemented in the PAUP software (*8*) for the nucleotide sequences obtained for the isolates and representative members of other Mayaro-related viruses belonging to the genus *Alphavirus* available at GenBank (www.ncbi.nlm.nih.gov). Bootstrap resample method (1,000 replicates) and outgoup definition were used to provide confidence for the phylogenetic groups (*9*).

The 3 MAYV isolates were successfully sequenced, and the nucleotide sequences covering the 3′ E1 region, the entire E2 gene, and 3′ noncoding region (≈2,000 nt) were phylogenetically compared with other MAYV and Mayaro-related viruses isolated during different periods (1954–2008) and from different hosts (human and arthropods) in Brazil, Peru, French Guiana, Trinidad and Tobago, Suriname, and Bolivia (Figure 2).

The phylogram depicted a clear segregation of MAYV strains into 2 major groups: genotypes D and L (*1*). The strains isolated in Santa Barbara municipality were grouped together in genotype D within clade I. Genetically, these strains were closely related to a 1991 isolate from Tocantins state in northern Brazil. The strains isolated in Santa Barbara were similar to those isolated in Belém during the same period. Interestingly, the Santa Barbara and Belém strains differed from the Brazilian and prototype strains isolated in 1955 (Figure 2).

Conclusions

MAYV has been isolated only in northern South America. Probably because of the short viremic period, it is sporadically isolated only during enzootic periods. However, during epidemics or epizootics, the number of isolates increase sharply (*10,11*). The few isolates obtained are intriguing and contrast with the high prevalence of specific antibodies in Pan-Amazonia; previous studies have shown widespread immunity in the Amazon, ranging from 5% to 60%. Positivity increases with age and is higher in rural and neighboring communities, as observed for the Amerindians (*2,12,13*).

In a previous outbreak in Belterra, several patients were too ill to continue their daily activities while febrile, and some even became prostrate. Moreover, these patients frequently reported severe arthralgia that led to temporary incapacitation (*13,14*).

Our data confirmed the occurrence of a Mayaro fever outbreak in the Pau D'Arco settlement. Clinically, the disease was similar to other outbreaks and characterized mainly by fever, arthralgia, myalgia, headache, rash, and dizziness (*2,13–15*). This outbreak was reported 17 years after the last episode of the disease described in the municipality of Benevides, which is closer (≈10 km) to Santa Barbara (P.F.C. Vasconcelos, unpub. data). The clinical and laboratory data from this MAYV outbreak caused by genotype D confirmed in Santa Barbara provide a better understanding of the MAYV molecular epidemiology in the Brazilian Amazon region.

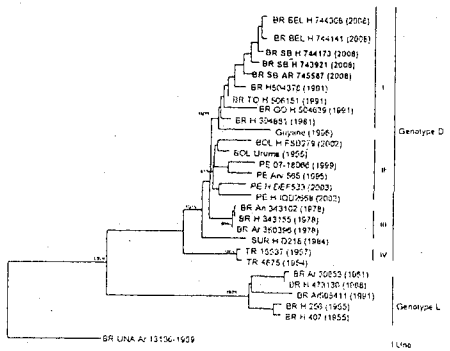


Figure 2. Comparison of genetic relationships among the Mayaro virus strains sequenced in this study with those isolated in different areas of South America, periods of time, and hosts. Numbers above and within parentheses correspond to bootstrap support values for the specific clades. The Una virus was used as an outgroup to root the tree. BR, Brazil (BEL, Belém; SB, Santa Barbara [bold]; TO, Tocantins state); BOL, Bolivia; PE, Peru; SUR, Suriname; H, human; Ar, arthropod. Numbers in parentheses correspond to the year of isolation of each strain. Items in **boldface** indicate strains isolated in this study.

Acknowledgments

We thank Basílio Buna, Creuza Carvalho, Hélio Saraiva, Luiz Roberto Costa, and Orlando Vaz da Silva for their technical assistance.

This work was supported by Instituto Evandro Chagas/Secretaria de Vigilância em Saúde/Ministry of Health and Conselho Nacional para o Desenvolvimento Científico e Tecnológico (grants 300460/2005-8 and 302987/2008-8).

Dr Azevedo is a physician working with arboviruses and rodent-borne viruses at Instituto Evandro Chagas. Her research interests include epidemiology of these and other emerging infectious diseases.

References

1. Powers AM, Aguilar PV, Chandler LJ, Brault AC, Meakins TA, Watts D, et al. Genetic relationships among Mayaro and Una viruses suggest distinct patterns of transmission. Am J Trop Med Hyg. 2006;75:461–9.
2. Pinheiro FP, LeDuc JW. Mayaro virus disease. In: Monath TP, editor. The arboviruses: epidemiology and ecology. Vol 3. Boca Raton (FL): CRC Press; 1988. p. 137–50.
3. Kuno G, Gomez I, Gubler DJ. Detecting artificial anti-dengue IgM *immune complexes* using an enzyme-linked immunosorbent assay. Am J Trop Med Hyg. 1987;36:153–9.
4. Beaty B, Calisher CH, Shope RE. Arboviruses. In: Lennette EH, Lennette DA, Lennette ET, editors. Diagnostic procedures for viral, rickettsial and chlamydial infections. 7th ed. Washington: American Public Health Association; 1995. p. 189–212.
5. Tesh RB. A method for the isolation and identification of dengue viruses, using mosquito cell cultures. Am J Trop Med Hyg. 1979;28:1053–9.
6. Sanger F, Nicklen S, Coulson AR. DNA sequencing with chain-terminating inhibitors. Proc Natl Acad Sci U S A. 1977;74:5463–7. DOI: 10.1073/pnas.74.12.5463
7. Kimura M. A simple method for estimating evolutionary rate of base substitution through comparative studies of nucleotide sequences. J Mol Evol. 1980;16:111–20. DOI: 10.1007/BF01731581
8. Swofford DL. PAUP*. Phylogenetic analysis using parsimony (*and other methods), version 4. Sunderland (MA): Sinauer Associates; 1999.
9. Felsenstein J. Confidence limits on phylogenies: an approach using the bootstrap. Evolution. 1985;39:783–91. DOI: 10.2307/2408678
10. Vasconcelos PF, Travassos da Rosa AP, Dégallier N, Travassos da Rosa JF, Pinheiro FP. Clinical and ecoepidemiological situation of human arboviruses in Brazilian Amazonia. Braz J Assoc Advanc Sci. 1992;44:117–24.
11. Vasconcelos PF, Travassos da Rosa AP, Pinheiro FP, Shope RE, Travassos da Rosa JF, Rodrigues SG, et al. Arboviruses pathogenic for man in Brazil. In: Travassos da Rosa AP, Vasconcelos PF, Travassos da Rosa JF, editors. An overview of arbovirology in Brazil and neighbouring countries. Belém (Brazil): Instituto Evandro Chagas; 1998. p. 72–99.
12. Theiler M, Downs WG. The arthropod-borne viruses of vertebrates. New Haven (CT): Yale University Press; 1973. p. 131.
13. Pinheiro FP, Freitas RB, Travassos da Rosa JF, Gabbay YB, Mello WA, LeDuc JW. An outbreak of Mayaro virus disease in Belterra, Brazil. I. Clinical and virological findings. Am J Trop Med Hyg. 1981;30:674–81.
14. LeDuc JW, Pinheiro FP, Travassos da Rosa AP. An outbreak of Mayaro virus disease in Belterra, Brazil. II. Epidemiology. Am J Trop Med Hyg. 1981;30:682–7.
15. Tesh RB. Arthritides caused by mosquito-borne viruses. Annu Rev Med. 1982;33:31–40. DOI: 10.1146/annurev.me.33.020182.000335

Address for correspondence: Pedro F.C. Vasconcelos, Instituto Evandro Chagas, Rodovia BR 316, KM 7, CEP 67030-000, Ananindeua, Pará, Brazil; email: pedrovasconcelos@iec.pa.gov.br

別紙様式第2-1

No. 10

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
				2010. 1. 26	該当なし	
一般的名称	人血清アルブミン		研究報告の公表状況	Pecorari M, Longo G, Gennari W, Grottola A, Sabbatini A, Tagliazucchi S, Savini G, Monaco F, Simone M, Lelli R, Rumpianesi F. Euro Surveill. 2009 Dec 17;14(50). pii: 19446.	公表国 イタリア	
販売名(企業名)	赤十字アルブミン20(日本赤十字社) 赤十字アルブミン25(日本赤十字社) 赤十字アルブミン20%静注4g/20mL(日本赤十字社) 赤十字アルブミン20%静注10g/50mL(日本赤十字社) 赤十字アルブミン25%静注12.5g/50mL(日本赤十字社)					

研究報告の概要

○ウスツウイルス神経浸潤感染の初のヒト症例:イタリア2009年8～9月

ウスツウイルス(USUV)はフラビウイルス科の節足動物媒介性ウイルスで、日本脳炎ウイルスと近縁である。過去10年間に、中央ヨーロッパで脳炎、心筋変性、肝臓・脾臓壊死を発症した鳥類から検出された。これまでにヒトにおいて重症もしくは死に至る疾患を発症した例はなかった。ここでは、発熱と神経症状を呈し髄膜脳炎と診断された、世界初となるUSUV神経浸潤感染の症例を報告する。

患者はイタリア在住の60代女性で、2009年5月にびまん性大細胞型B細胞リンパ腫のため半結腸切除術を受けた。同年8月21日までに6回の化学療法を受けた。その数日後、性器ヘルペスの再燃がありバラシクロビルを投与された。9月1日に39.5℃の発熱と安静時振戦を発症し、抗菌薬を投与されたものの高熱は持続した。9月5日に高熱と静注抗菌薬治療のため入院し、重度の貧血のため輸血を受けた。9月11日に採取された脳脊髄液はUSUV陽性であり、RT-PCRと塩基配列決定によって血清および血漿検体にもUSUVが示された。premembraneの部分配列とウイルス-ゲノムのNS5領域は、USUV Vienna株およびBudapest株と相同していた。髄膜脳炎の急性期前と後の血清(5月26日、10月13日)及び血漿(10月19日)検体からはウイルスは検出されなかった。

使用上の注意記載状況・その他参考事項等

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

血液を原料とすることに由来する感染症伝播等

報告企業の意見	今後の対応
発熱と神経症状を呈し髄膜脳炎と診断された、世界初となるウスツウイルス神経浸潤感染の症例報告である。 ウスツウイルスは脂質膜を持つ中型のRNAウイルスで、これまで本製剤によるウスツウイルス感染症の報告はない。本製剤の製造工程には、平成11年8月30日付医薬発第1047号に沿ったウイルス・プロセスバリデーションによって検証された2つの異なるウイルス除去・不活化工程が含まれていることから、本剤の安全性は確保されていると考える。	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(入国)後4週間は献血不適としている。また、発熱などの体調不良者を献血不適としている。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。

32

6

MedDRA/J Ver.12.0J

JRC2010T-001

Rapid Communications

First human case of Usutu virus neuroinvasive infection, Italy, August-September 2009

M Pecorari (pecorari.monica@policlinico.mo.it)[1], G Longo[2], W Gennari[1], A Grottola[1], A MT Sabbatini[1], S Tagliazucchi[1], G Savini[3], F Monaco[3], M L Simone[3], R Lelli[3], F Rumpianesi[1]

1. Department of Diagnostic and Laboratory Services and Legal Medicine, University of Modena and Reggio Emilia, Azienda Ospedaliera Policlinico, Modena, Italy
2. Department of Oncology and Hematology, University of Modena and Reggio Emilia, Azienda Ospedaliera Policlinico, Modena, Italy
3. Department of Virology, National Reference Centre for West Nile and Usutu disease, OIE Reference Laboratory for Bluetongue, Istituto Zooprofilattico Sperimentale dell'Abruzzo e Molise 'G. Caporale', Teramo, Italy
4. Department of Biomedical Sciences, University of Modena and Reggio Emilia, Modena, Italy

This article was published on 17 December 2009.
Citation style for this article: Pecorari M, Longo G, Gennari W, Grottola A, Sabbatini AM, Tagliazucchi S, Savini G, Monaco F, Simone ML, Lelli R, Rumpianesi F. First human case of Usutu virus neuroinvasive infection, Italy, August-September 2009. Euro Surveill. 2009;14(50):pii=19446. Available online: http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=19446

We report the first worldwide case of Usutu virus (USUV) neuroinvasive infection in a patient with diffuse large B cell lymphoma who presented with fever and neurological symptoms and was diagnosed with meningoencephalitis. The cerebrospinal fluid was positive for USUV, and USUV was also demonstrated in serum and plasma samples by RT-PCR and sequencing. Partial sequences of the premembrane and NS5 regions of the viral genome were similar to the USUV Vienna and Budapest isolates.

Introduction

Usutu virus (USUV) is an arthropod-borne virus of the family *Flaviviridae*, genus *Flavivirus*. It is included in the Japanese encephalitis virus (JEV) group [1] being closely related to human pathogens such as JEV and West Nile virus (WNV). In the last decade, USUV was detected in a variety of central European birds with encephalitis, myocardial degeneration, and necrosis in liver and spleen [2-5]. As far as we know, the virus had never been associated with severe or fatal disease in humans [6]; it was isolated once in the Central African Republic in a man with fever and rash [7]. Here we report evidence of a neuroinvasive infection clinically related to USUV in Italy.

Case report

In May 2009, a woman in her 60s from Emilia Romagna region, Italy, underwent hemicolectomy because of a diffuse large B cell lymphoma. Six courses of chemotherapy were administered (including rituximab, cyclophosphamide, doxorubicin, vincristine and prednisone), with last administration on 21 August 2009. Some days later, there was a reactivation of genital herpes treated with valacyclovir. On 1 September, a fever of 39.5°C with resting tremor appeared and antibiotic (moxifloxacine and amoxicilline clavulanate) therapy started however the temperature persisted. On 5 September, the patient was admitted to hospital for hyperpyrexia resistant to antipyretic and intravenous antibiotic treatment (meropenem and teicoplanine). Once admitted, the patient received blood transfusion because of a critical anaemia.

Examination of blood, urine and stool cultures and virological assessment for herpes virus simplex (HSV1/2) and cytomegalovirus (CMV) antigen were negative. A total body computerised tomography was performed without evidence of lymphoma. Suspicion of meningoencephalitis was addressed by neurological examination which showed distal resting tremor, positivity to the Romberg test, dysmetry and weakness at four limbs without cranial nerve affection. Magnetic resonance imaging (MRI) of the brain showed a signal alteration of the substantia nigra of the parietal and frontal subcortical areas that did not change after injection of contrast medium. On 11 September, the cerebrospinal fluid (CSF) was therefore collected and examined. The CSF was limpid without any alteration detected in the clinical-chemical analysis, activated lymphocytes were evident in the sediment. As further analysis of the same CSF specimen revealed the presence of flaviviruses (see below), steroid treatment was started. This therapy resolved the fever but did not lead to any improvement of the neurological symptoms. The electroencephalogram still registered diffuse slow theta waves and slow spike prevalent in left frontal parietal area. The neurological functions, mainly the resting tremor, improved following the administration of levodopa and carbidopa.

Virological analysis

When tested for the presence of viral agents, the CSF collected on 11 September was negative in molecular tests for CMV, HSV1/2, Epstein-Barr virus, adenoviruses, parvovirus B19, polyomavirus JC and BK, enteroviruses, mumps virus and WNV and positive to a heminested RT-PCR specific for the NS5 region of the *Flavivirus* genus [8]. The amplicon was directly sequenced and analysed by BLAST (http://www.ncbi.nlm.nih.gov/blast), revealing a 98% identity with both the USUV Budapest (gbIEF206350.1) and Vienna (gbJAY453411.1) isolate.

To confirm the identification of the species Usutu virus, we performed two USUV-specific RT-PCRs targeting the NS5 [2] and premembrane (preM) regions (primer sequences available on request) of the USUV genome on two plasma specimens collected on 8 and 11 September 2009 and one serum specimen collected on 14 September. The amplified products were sequenced (583 bp of NS5 and 602 bp of preM) and aligned with the corresponding sequences deposited in Genbank (gbJAY453411.1;

gbIEF206350.1) using ClustalW. The alignment of the preM gene shared 99% nucleotide identity with the USUV Budapest and Vienna sequences, whereas the NS5 gene sequences shared 100% nucleotide identity with USUV Vienna and 99% with USUV Budapest.

Further specimens of serum (26 May and 13 October) and plasma (19 October) before and after the acute phase of meningoencephalitis were analysed to demonstrate the absence of the virus. The two USUV-specific RT-PCRs performed on these three samples did not detect any USUV RNA. These samples were also analysed for WNV because a WNV outbreak was ongoing in the area at the time [9], and were negative.

Discussion

To our best knowledge this the first human disease with neurological involvement caused by USUV. The detection of USUV only in those samples collected during the acute phase of clinical manifestation is clear evidence that the virus caused e meningoencephalitis in the patient. Its capability of causing neurological lesions and death has already been reported in birds of central Europe [10]. The presence of USUV in Emilia Romagna has also been reported [4] and, in the past few months, the virus was isolated from black birds found dead in Northern Italy [G. Savini, personal communication 22 October 2009]. A surveillance programme in sentinel chicken flocks to monitor the possible appearance and/or circulation of WNV and other flaviviruses has been in place for several years. In the clinical case reported here, the immunosuppressed status of the patient due to both the underlying disease and the treatment, particularly with rituximab, may have played an important role in USUV infection and in its pathogenicity. It is known that rituximab can reactivate hepatitis B virus in patients with lethal fulminant hepatitis.

However, a possible unusual neuroinvasiveness and neurovirulence of this particular USUV strain cannot be excluded. The fact that neurological symptoms occurred prior to hospital admission excludes the transfusion as a possible source of infection. Conversely, since USUV as well as competent viral vectors are circulating in the patient's area of residence [4], it is likely that the infection was transmitted to the patient through mosquito bites.

References

1. Heinz FX, Collett MS, Purcell RH, Gould EA, Howard CR, Houghton RJ, et al. Family Flaviviridae. Virus Taxonomy. Seventh Report on International Committe on Taxonomy of Viruses.van Regenmortel MHC, Fauquet CM, Bishop DHL, Carstens EB, Estes MK, Lemon SM, et al, editors. San Diego, San Francisco, New York, Boston, London, Sydney, Tokyo: Academic Press; 2000. p. 859-78.
2. Bakonyi T, Erdélyi K, Ursu K, Ferenczi E, Csörgo T, Lussy H, et al. Emergence of Usutu virus in Hungary. J Clin Microbiol. 2007;45(12):3870-4.
3. Chvala S, Kolodziejek J, Nowotny N, Weissenböck H. Pathology and viral distribution in fatal Usutu virus infections of birds from the 2001 and 2002 outbreaks in Austria. J Comp Pathol. 2004;131(2-3):176-85.
4. Lelli R, Savini G, Teodori L, Filipponi G, Di Gennaro A, Leone A, et al. Serological evidence of USUTU virus occurrence in north-eastern Italy. Zoonoses Public Health. 2008; 55(7): 361-7.
5. Manarolla G, Bakonyi T, Gallazzi D, Crosta L, Weissenböck H, Dorrestein GM, et al. Usutu virus in wild birds in northern Italy.Vet Microbiol. 2009; Aug 8.
6. Weissenböck H, Hubálek Z, Bakonyi T, Nowotny N. Zoonotic mosquito-borne flaviviruses: Worldwide presence of agents with proven pathogenicity and potential candidates of future emerging diseases. Vet. Microbiol. 2009; Aug 26.
7. Adam F, Diguette J-P. Virus d'Afrique [base de données].[Internet]. Dakar: Institut Pasteur de Dakar. Centre collaborateur OMS de référence et de recherche pour les arbovirus et les virus de fièvres hémorrhagiques (CRORA).. Available from: http://www.pasteur.fr/recherche/banques/CRORA
8. Scaramozzino N, Crance JM, Jouan A, Debriel DA, Stoll F, Garin D. Comparison of Flavivirus universal primer pairs and development of a rapid, highly sensitive heminested reverse transcription-PCR assay for detection of flaviviruses targeted to a conserved region of the NS5 gene sequences. J. Clin. Microbiol. 2001;39(5):1922-7.
9. Rizzo C, Vescio F, Declich S, Finarelli AC, Macini P, Mattivi A, et al. West Nile virus transmission with human cases in Italy, August - September 2009. Euro Surveill. 2009;14(40):pii=19353. Available from: http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=19353
10. Weissenböck H, Kolodziejek J, Url A, Lussy H, Rebel-Bauder B, Nowotny N. Emergence of Usutu virus, an African mosquito-borne flavivirus of the Japanese encephalitis virus group, central Europe. Emerg Infect Dis. 2002;8(7):652-6.

別紙様式第 2-1

医薬品
医薬部外品　研究報告　調査報告書
化粧品

別紙 3-1

識別番号・報告回数		回	報告日 年 月 日	第一報入手日 2009 年 9 月 4 日	新医薬品等の区分 該当なし	総合機構処理欄
一般的名称				Effectiveness of nanofiltration in removing small non-enveloped viruses from three different plasma-derived products. M. C. Menconi et al., Transfusion Medicine, 2009, 19, 213- 217	公表国 イタリア	
販売名（企業名）			研究報告の公表状況			使用上の注意記載状況・その他参考事項等 BYL-2010-0395
研究報告の概要	血漿由来製剤は世界中の多くの患者にとって重要な治療薬である。これらの製造過程において，感染性病原体による汚染を防止するため，ウイルスの不活性化と除去処理を用いた効果的なウイルス除去工程が導入されている。ナノフィルトレーション（ウイルス除去膜濾過）は特にサイズ排除によるウイルス除去構造となっており，ヒトパルボウイルス B19（B19V）やトルクテノウイルス（TTV）などの小型でエンベロープを持たないウイルスを除去する際に有効とされている。本稿では，3 種類の血漿由来製剤：アルブミン溶液，プロトロンビン複合体（PTC），血液凝固第 IX 因子（FIX）から B19V および TTV をナノフィルトレーションによって除去し，その効果を評価した。各製剤に各ウイルス DNA 陽性血清を添加し，孔径 0.22 μm のプレフィルターで前処理した後，孔径 35 nm および 15 nm のプラバノ・フィルターによる末端濾過方式による定圧濾過を実施した。ウイルス除去効率を計測するためのウイルス量の測定はリアルタイム PCR 法を用いた。15 nm の濾過膜処理の結果，全ての製剤において B19V については 4.0 $\log_{10}$ 以上の除去能が確認された。TTV は，アルブミン溶液および FIX において 3.0 $\log_{10}$ 以上が除去されたが，PTC において 15 nm の濾過膜処理後も高い除去効率は得られなかった。以上より，ナノフィルトレーションは血漿由来製剤のウイルス除去に有効であると考えられるが，似たようなウイルスであっても，溶液中のタンパクの組成構造やタンパク濃度により除去効率が影響を受けることが示唆された。				
	報告企業の意見		今後の対応			
	B19V では，本研究で行われた孔径のナノフィルトレーションにより除去可能であることが判明したが，現時点では濾過に用いた容量が小さいため，今後より大量の溶液を用いての除去検査が必要であると考える。一方，TTV の場合には，ナノフィルトレーションの効果が最大限発揮できる PTC 濃度の調整が必要である。なお，弊社のコージネイト FS およびコージネイト FS バイオセットの製造工程培地で使用されている血漿分画成分に使用されるミニプール血漿においては，ヒトパルボウイルス B19 に対する NAT を実施しており，10E5 IU/mL 以上が確認された場合は，そのミニプール血漿は製造工程から除去している。製造工程においては，非エンベロープ 1 本鎖 DNA ウイルスについてブタパルボウイルスをモデルとした除去効率が 2.1log であることが実証されている。弊社で使用している血漿タンパクは培地成分としての使用であるため，伝播の可能性は非常に低いと考える。	現時点で新たな安全対策上の措置を講ずる必要はないと考えるが，今後ともウイルス除去特にヒトパルボウイルス B19 といった小型非エンベロープウイルスの除去効率の改善に関する情報収集に努める。		

Transfusion Medicine, 2009, 19, 213–217
doi: 10.1111/j.1365-3148.2009.00931.x

SHORT COMMUNICATION

Effectiveness of nanofiltration in removing small non-enveloped viruses from three different plasma-derived products

M. C. Menconi,* F. Maggi,† K. Zakrzewska,‡ V. Salotti,§ P. Giovacchini,* C. Farina,* E. Andreoli,† F. Corcioli,‡ M. Bendinelli,† & A. Azzi‡ • *Kedrion S.p.A. Research Centre, Castelvecchio Pascoli (LU); †Virology Section and Retrovirus Center, Department of Experimental Pathology, University of Pisa, Pisa, ‡Department of Public Health, University of Florence, Florence, and §Genedia Srl, R&D, Napoli, Italy*

Received 03 December 2008; accepted for publication 06 February 2009

SUMMARY. The objective of this study was to assess the ability of nanofiltration of albumin solution, prothrombin complex (PTC) and factor IX (FIX) to remove two small, non-enveloped DNA viruses, parvovirus B19 (B19V) and torque teno virus (TTV). Virus removal was investigated with down-scale experiments performed with sequential steps of 35-nm and 15-nm nanofiltrations of products spiked with virus DNA-positive sera. Viral loads were determined by real-time PCRs. The 15-nm nanofiltration removed more than 4.0 B19V log from all the products, TTV was reduced of more than 3.0 log from albumin solution and FIX by 35-nm and 15-nm nanofiltrations, respectively, being viral DNA undetectable after these treatments. Traces of TTV were still found in PTC after the 15-nm nanofiltration. In conclusion, nanofiltration can be efficacious in removing small naked viruses but, since viruses with similar features can differently respond to the treatment, a careful monitoring of large-scale nanofiltration should be performed.

Key words: albumin solution, factor IX, nanofiltration, plasma-derived products, parvovirus B19, prothrombin complex, TTV.

INTRODUCTION

Plasma-derived proteins are important therapeutics for many patients all over the world. In order to prevent the contamination of these products by infectious agents, special care is paid to avoid the collection of contaminated plasma units by donor selection and plasma donations testing for markers of infections. In addition, robust and validated viral clearance steps using inactivation and removal treatments are included in the manufacturing process (The European Agency, 2001; Burnouf & Radosevich, 2003; World Health Organization, 2004).

Nanofiltration is specifically designed to remove viruses through a size exclusion mechanism. Several studies, performed using plasma-borne and model viruses, show that a nanofiltration typically allows up to four to six logs of virus removal, depending upon the membrane used, under conditions that ensure good protein permeability and recovery (Troccoli *et al.*, 1998; Chandra *et al.*, 2002). Nanofiltration should be particularly useful in removing some viruses, such as the small non-enveloped viruses like human parvovirus B19 (B19V) and torque teno virus (TTV). Actually, the two common viral plasma contaminants (Maggi *et al.*, 2003; Azzi *et al.*, 2006) have shown to be difficult to inactivate/remove by conventional physico-chemical treatments (Omar & Kempf, 2002; Yokoyama *et al.*, 2004; Kreil *et al.*, 2006), even if recent findings have pointed out a higher vulnerability of B19V in comparison to some animal parvovirus (Blumel *et al.*, 2002; Blumel *et al.*, 2008; Boschetti *et al.*, 2004; Mani *et al.*, 2007; Berting *et al.*, 2008).

In addition to B19V, many TTV characteristics led to concerns about the potential for its transmission and pathogenicity in humans by contaminated plasma-derived products and other biopharmaceutical agents. TTV is the prototype of related yet clearly distinct viruses currently classified in the newly established genus *Anellovirus*. The virus is characterized by several well-known properties: (i) a particularly small (about 3.7 kb) single-stranded circular DNA genome characterized by an extremely high degree of genetic heterogeneity; (ii) a remarkable ability to produce persistent infections in the general population worldwide (about 90% of individuals carry TTV DNA in their blood), with variably elevated levels of plasma viraemia (from 10^2 to 10^8 DNA copies per ml); (iii) a general ubiquity in the body where it replicates very actively in most tissues and organs (Maggi and Bendinelli, 2009; Okamoto, 2009).

In this study, we evaluate the efficacy of nanofiltration in removing B19V and TTV from three plasma-derived products: albumin solution, prothrombin complex (PTC) and factor IX (FIX).

Correspondence: Alberta Azzi Department of Public Health, University of Florence, Viale Morgagni 48 50134 Firenze, Italy.
Tel.: +39 055 4598557; fax: +39 055 4598924
e-mail: alberta.azzi@unifi.it

MATERIALS AND METHODS

The three products used in this study were sampled from their respective bulk solutions. Albumin bulk solution was obtained from raw Fraction V after Cohn fractionation of plasma; PTC bulk solution was purified from plasma cryo pool with a double anionic exchange chromatography (Brummelhuis, 1980; Josic *et al.*, 2000); FIX bulk solution was purified from plasma cryo pool in two chromatographic steps, an anionic exchange followed by affinity chromatography on Heparin Sepharose (Michalski *et al.*, 1988). The samples, previously frozen, had different protein concentration, as reported in Table 1. Albumin solution purity was 97% and in FIX the coagulation factor specific activity was 64.13 UI/mg. In PTC, the FIX specific activity was 3.48 UI/mg, factor II (FII) specific activity was 3.35 UI/mg and factor X (FX) specific activity was 2.7 UI/mg.

Two hundred ml of each product was thawed in a water bath at 37°C and homogenized by mechanical stirring. Human sera containing known numbers of viral genomes and kept as aliquots at −80°C were used as a source of B19V or TTV as both viruses fail to grow efficiently in tissue culture. For B19V, serum S22, obtained from a viremic patient and stocked at −80°C in small aliquots, which contained 1.0×10^{12} genome copies/ml and no detectable anti-B19V antibody, was used for all the spiking experiments at 1:100 dilution. For TTV, a positive serum, obtained from a healthy donor after blood centrifugation, was used containing 1.6×10^8 viral genomes per ml as determined by real-time polymerase chain reaction (PCR). The two sera were free of hepatitis B and C viruses and human immunodeficiency virus (HIV), as determined by specific serological and molecular assays.

The high virus titer of the two sera allowed the use of the minimum percent spike compatible with reaching a target reduction factor of 4, in order to minimize filters fouling by impurities of the virus stock preparations as well as the impact of the serum proteins on the composition of the examined bulk solutions.

Two hundred µl of each sera was spiked into each product and homogenized for 1 h. Once the 0.22 µm pre-filtration was done, each solution was filtered in a dead-end flow filtration mode through a 35-nm Planova filter (Asahi Chemical Industries, Japan) with an effective surface area of 0.01 m^2, followed by a 15-nm Planova filter with the same surface area, at a constant pressure of 0.5 bar. When the flow of filtered material decreased below 0.4-0.5 ml/min, the pressure was increased up to a maximum of 0.8 bar. From the starting products as well as after each filtration, samples were collected for protein titre and protein activity (only for PTC and FIX) determination and for viral quantitation.

Protein measurements were performed according to Bradford (Bradford, 1976) with an ultraviolet/visible (UV/VIS) Lambda 1A spectrophotometer (PerkinElmer, MA, USA). FIX activity was estimated with a one-stage coagulation assay on ACL 7000 (Instrumentation Laboratory, Spain); FII and FX were still evaluated on ACL 7000 but with a chromogenic assay.

The presence and the loads of B19V DNA were determined by a real time PCR (Real Quant B19 KIT, GeneDia, Naples, Italy; Azzi *et al.*, 2006) with a detection limit of 100 DNA copies/ml of serum and range of linearity 10^2–10^7. TTV quantitation was performed

Table 1. Volume and protein titre variations recorded during the overall filtration process

	Starting volume (ml)	Post 0.22 µm (ml)	Post 35 nm (ml)	Post 15 nm (ml)
Albumin	200	192	179	161
PTC	198	198	194	192
FIX	200	200	198	192
	Starting protein titre (mg/ml)	Post 0.22 µm (mg/ml)	Post 35 nm (mg/ml)	Post 15 nm (mg/ml)
Albumin	91.39	87.56	88.43	85.47
PTC	1.207	1.194	1.120	0.757
FIX	0.219	0.216	0.213	0.172

by using a single step universal TaqMan real-time PCR assay as described previously (Maggi *et al.*, 2001; Maggi *et al.*, 2003). Its lower limit of detection was 1000 DNA copies/ml of serum. Each PCR run contained several negative control (no template) as well as the reference template (positive control) at 10^1–10^7 DNA copies/10 µl. Both controls and samples were tested in triplicates. All samples positive in only one replicate and/or with a coefficient of variation of 50% or greater were re-extracted and tested again in triplicate. Since theoretically samples tested negative in all PCR replicates couldn't be completely virus-free, according to the sensitivity limit of the assays used they were indicated as having less than 2.0 and 3.0 log of B19V and TTV DNA, respectively.

All experiments (one for albumin, one for FIX, three for PTC) were performed on different days under a laminar flow hood equipped with UV light. Moreover, all the necessary steps to avoid the risk of carry-over PCR contamination of samples were also taken.

The PTC solution after 15-nm filtration was treated with DNase I (200U/ml, Roche, Mannheim, Germany) for 2 h (Azzi *et al.*, 2006) and examined again for TTV DNA levels.

RESULTS

Protein and activity recovery

The volume and the protein titre measured at the end of each filtration step are shown in Table 1. A quite marked volume loss (19.5%) was observed at the end of the overall filtration process of the albumin solution and was mainly due to an early stop of each filtration in order to avoid foaming of the solution. The protein content decrease in the albumin solution was not significant and the reduction of protein content (24.71%) was mostly due to material loss.

Volume loss of PTC and FIX was negligible (3–4%). On the contrary, after 15-nm filtration, the protein content decrease was 39.2% and 24.6% for PTC and FIX, respectively. The decrease of PTC protein content correlated with a marked loss of FIX (36.7%) and FII (30.4%) activity, whereas the coagulation factor activity was not so strongly modified by nanofiltration of FIX (7.5% of loss). By nephelometric assay, it was verified that at least high molecular weight protein C4 was present in FIX and was reduced after the 15-nm filtration (56% reduction). Thus, the 15-nm filtration of FIX seems to increase the purity of the active substance.

Viral clearance

Post spiking, B19V loads varied from 7.5 to 6.9 $\log_{10}$ copies/ml in different products (Table 2). The pre-filtration step removed less than one $\log_{10}$ of B19V DNA from the spiked products. The first nanofiltration step further reduced the viral load by 0.4 to 1.2 $\log_{10}$ and after the 15-nm filtration, B19V DNA was undetectable in all the products.

Post-spiking contaminating TTV varied from 5.0 to 6.3 $\log_{10}$ copies/ml in the different products. The 0.22 µm filtration reduced 1.0 $\log_{10}$ of the starting TTV levels from albumin solution, while no or very slight reduction of TTV was observed from FIX and PTC. The subsequent nanofiltration successfully contributed to the removal of TTV. Albumin solution yielded no detectable TTV already, after 35-nm filtration, whereas a 15-nm filtration was required for FIX. Unexpectedly, the residual TTV DNA (approximately 2.5% of the post-spiking content) was still detectable in PTC after the 15-nm filtration (Table 2). To shed light on the latter finding, two further experiments of PTC nanofiltration were performed with conflicting results: in one experiment, no TTV DNA was detectable, whereas, in the other, traces of viral DNA were still found after the 15-nm filtration (data not shown).

TTV detection in the PTC solution after the 15-nm filtration and DNase treatment revealed no variation of virus loads, thus excluding the presence of naked DNA.

Table 2. Removal of B19V and TTV by sequential nanofiltration

	Post-spiking viral load ($\log_{10}$ DNA copies/ml)	B19V and TTV DNA recovery*		
		Post 0.22 µm	Post 35 nm	Post 15 nm
Albumin				
B19V	6.9	6.5	5.3	<2.0
TTV	6.3	5.3	<3.0	<3.0
PTC				
B19V	7.5	6.8	6.4	<2.0
TTV†	5.0	4.9	4.7	3.4
FIX				
B19V	7.4	7.3	6.6	<2.0
TTV	6.1	6.1	6.0	<3.0

*Log_{10} genome copies/ml recovered after each filtration step.
†Data from one experiment only are shown.

DISCUSSION

In our study, a blood-product with high protein concentration, such as albumin solution, was successfully nanofiltered at 35–15 nm. Anyway, an accurate set-up of the process should minimize the material loss in order to regard nanofiltration as a further step of viral removal in the albumin production.

Nanofiltration was successful for FIX, as the process slightly increased its purity. Besides, Hoffer *et al.* (1995) already found that high molecular mass impurities are retained by nanofilter membranes, resulting in increased FIX specific activity.

On the contrary, in spite of the fairly good filterability of PTC, the protein recovery, after the 15-nm filtration, as well as the recovered FIX and FII activities, was unsatisfactory. On the other hand, as previously described (Josic *et al.*, 2000), the high molecular weight components of PTC could form protein complexes with the coagulation factors, thus hindering their filtration.

In regard to the nanofiltration ability in removing infectious agents from the above blood products, the behaviour of two small non-enveloped viruses, B19V and TTV, was not completely identical. Although mostly based on individual experiments, no detectable B19V was found in the three products following 15-nm nanofiltration, whereas TTV was totally cleared only from the albumin solution and FIX by 35-nm and 15-nm nanofiltrations, respectively. Interestingly, low levels of TTV DNA (less than 3000 copies per ml) were still present in PTC after the 15-nm nanofiltration step in two of three experiments.

Although the serum used as B19V positive inoculum was anti-B19V antibody free, serum samples used for TTV spiking contained anti-TTV activity (Kreil *et al.*, 2006). The presence of TTV-antibody complexes, increasing the effective virus size, could explain the complete virus removal from the albumin solution by a 35-nm nanofiltration. In addition, the high protein concentration of this solution could have formed a protein layer on filter surfaces with a partial block of the small filter pores. Indeed, only a small amount of TTV was removed by a 35-nm filtration of a 0.25 g/l FIX solution and of a 1.5 g/l PTC solution. The complexity of PTC composition and the characteristics of TTV are likely responsible for the behaviour described concerning PTC nanofiltration, but further studies are necessary to understand the basis of such a peculiar behaviour better. To this purpose, it could be particularly relevant to investigate whether the TTV nanofiltration may be influenced by changes in the protein concentration of PTC, as our conflicting results seem to suggest. However, as previously reported (Kreil *et al.*, 2006), it is highly unlikely that a viral load as high as that used in our experiments may still be present in PTC after the use of all procedures for viral inactivation/elimination. Thus, on the basis of our results, it is to be expected that a low concentration of TTV, possibly residual post-PTC purification, should be easily removed by nanofiltration.

This study was supported by grant S454/P from the Ministero dell'Istruzione, dell'Università e della Ricerca, Rome, Italy.

REFERENCES

Azzi, A., Maggi, F., Zakrzewska, K. *et al.* (2006) Different behavior of erythrovirus B19 and torquetenovirus in response to a single step of albumin purification. *Transfusion*, **46**, 1162–1167.

Berting, A., Modrof, J., Unger, U., Gessner, M., Klotz, A., Poelsler, G. & Kreil, TR. (2008) Inactivation of parvovirus B19 during STIM-4 vapor heat treatment of three coagulation factor concentrates. *Transfusion*, **48**, 1220–1226.

Blümel, J., Schmidt, I., Willkommen, H.& Löwer, J. (2002) Inactivation of parvovirus B19 during pasteurization of human serum albumin. *Transfusion*, **42**, 1011–1018.

Blümel, J., Stühler, A. & Dichtelmüller, H. (2008) Kinetics of inactivating human parvovirus B19 and porcine parvovirus by dry-heat treatment. *Transfusion*, **48**, 790.

Boschetti, N., Niederhauser, I., Kempf, C., Stühler, A., Löwer, J. & Blümel, J. (2004) Different susceptibility of B19 virus and mice minute virus to low pH treatment. *Transfusion*, **44**, 1079–1086.

Bradford, MM. (1976) A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Analytical Biochemistry*, **72**, 248–254.

Brummelhuis, HGJ. (1980) Preparation of the prothrombin complex. In: *Methods of Plasma Proteins Fractionation* (ed. Curling, JM), 117–128. Academic Press, London.

Burnouf, T. & Radosevich, M. (2003) Nanofiltration of plasma-derived biopharmaceutical products. *Haemophilia*, **9**, 24–37.

Chandra, S., Groener, A., & Feldman, F. (2002) Effectiveness of alternative treatments for reducing potential viral contaminants from plasma-derived products. *Thrombosis Research*, **105**, 391–400.

The European Agency for the Evaluation of Medicinal Products, Evaluation for Medicines for Human Use (2001) Note for guidance on plasma-derived medicinal products. CPMP/BWP/269/95 (rev.3). Available at: http//www.emea.eu.int/pdfs/human/bwp/ 026995en.pdf.

Hoffer, L., Schwinn, H., Biesert, L., & Josic, D. (1995) Improved virus safety and purity of a chromatographically

virus inactivation and comparison to other parvoviruses. *Transfusion*, **47**, 1765–1774.
Michalski, C., Bal, F., Burnouf, T. & Goudemand, M. (1988) Large-scale production and properties of a solvent-detergent-treated Factor IX concentrate from human plasma. *Vox Sanguinis*, **55**, 202–210.
Okamoto, H. (2009) History of discoveries and pathogenicity of TT viruses. *Current Topics in Microbiology and Immunology*, **331**, 1–20.
Omar, A., & Kempf, C. (2002) Removal of neutralized model parvoviruses and enteroviruses in human IgG solutions by nanofiltration. *Transfusion*, **42**, 1005–1010.
Troccoli, NM., McIver, J., Losikoff, A., & Polley, J. (1998) Removal of viruses from human intravenous immune globulin by 35 nm nanofiltration. *Biological*, **26**, 321–329.
World Health Organization (2004) Guidelines on viral inactivation and removal procedures intended to assure the viral safety on human blood plasma products. WHO Technical Report, Series No. 924, Annex 4. Available at: http://www.who.int/bloodproducts/publications/WHO_TRS_924_A4.pdf.
Yokoyama, T., Murai, K., Murozuka, T., Wakisaka, A., Tanifuji, M., Fujii, N. & Tomono, T. (2004) Removal of small non-enveloped viruses by nanofiltration. *Vox Sanguinis*, **86**, 225–229.
Maggi, F., Pifferi, M. Fornai, C. *et al.* (2003) TT virus in the nasal secretions of children with acute respiratory diseases: relations to viremia and disease severity. *Journal of Virology*, **77**, 2418–2425.
Maggi, F., Pistello, M., Vatteroni, ML. *et al.* (2001) Dynamics of persistent TT virus infection, as estimated in patients treated with interferon-alpha for concomitant hepatitis C. *Journal of Virology*, **75**, 11999–12004.
Mani, B., Gerber, M., Lieby, P., Boschetti, N., Kempf, C. & Ros, C. (2007) Molecular mechanism underlying B19 produced factor IX concentrate by nanofiltration. *Journal of Chromatography B: Biomedical Sciences and Applications*, **669**, 187–196.
Josic, D., Hoffer, L., Buchacher, A., *et al.* (2000) Manufacturing of a prothrombin complex concentrate aiming at low thrombogenicity. *Thrombosis Research*, **100**, 433–441.
Kreil, TR., Wieser, A., Berting, A. *et al.* (2006) Removal of small nonenveloped viruses by antibody-enhanced nanofiltration during the manufacture of plasma derivatives. *Transfusion*, **46**, 1143–1151.
Maggi, F., Bendinelli, M. (2009) Immunobiology of the Torque Teno Viruses and other anelloviruses. *Current Topics in Microbiology and Immunology*, **331**, 65–90.

別紙様式第2-1

No. 25

医薬品 研究報告 調査報告書

識別番号・報告回数		報告日	第一報入手日	初回製造等の区分	総合機構処理欄
			2010. 1. 26	該当なし	

一般的名称	人血清アルブミン	研究報告の公表状況	公表国	使用上の注意記載状況・その他参考事項等
販売名（企業名）	赤+ケアルブミン20（日本赤十字社） 赤+ケアルブミン25（日本赤十字社） 赤+ケアルブミン20%静注4g/20mL（日本赤十字社） 赤+ケアルブミン20%静注10g/50mL（日本赤十字社） 赤+ケアルブミン25%静注12.5g/50mL（日本赤十字社）	MMWR Morb Mortal Wkly Rep. 2010 Jan 22;59(2):34-7.	米国	赤+ケアルブミン20 赤+ケアルブミン25 赤+ケアルブミン20%静注4g/20mL 赤+ケアルブミン20%静注10g/50mL 赤+ケアルブミン25%静注12.5g/50mL

○黄熱ワクチン（YFワクチン）ワクチンの輸血関連伝播−2009年カリフォルニア州

米国では、サハラ以南のアフリカや中南米の常在地域への旅行者および兵士に対して、黄熱(YF)ワクチン接種を推奨している。米国赤十字は、ワクチン反応による上記リスクの理論上の感染伝播リスクがあるとして、YFワクチン接種者に対し、2週間は血液製剤への献血を控えるよう求めている。

供血4日前の3月27日にYFワクチン接種を受けた米軍現役訓練生より供血が行われたことが2009年4月10日に病院の血液バンクに報告された。供血延期非実施の確認、YFワクチンワクチンの輸血関連伝播を検討するための病院とCDCによる調査について報告する。

調査により、訓練生の7防接種時期が最近変更されたこと、予防接種を受けた者が供血時にそれを報告しなかったことが判明した。製剤は迅速に回収されたにも関らず、患者5名に6単位が輸血された。輸血後1ヶ月以内の受血者4名に副作用は一致するも臨床所見・検査値異常は確認されなかった。残り01名の患者（前立腺がん、末期B細胞性リンパ腫）はかえて死亡した。生存者4名のうち3名は、YFワクチンワクチンの抗体反応の証拠があった。本報告は、YFワクチンワクチンが輸血により伝播する可能性があるエビデンスを最近入手した。輸血による黄熱ワクチンワクチンの感染伝播のリスクは、例え照射血小板製剤であっても起こりうることが示された。放射線照射は感染リスクを低減させるものの、線量としては、黄熱ワクチンのワクチンウイルスを死滅させるには不十分であった。生存中の血液製剤の受血者5人のうち3人は、YFV-1gMと中和抗体を持っていた。早期産児ではgM抗体が検出されなかったのは、乳児の免疫システムの未熟性に起因するとも考えられた。

研究報告の概要

報告企業の意見

米国で供血4日前に黄熱ワクチン接種を受けた米軍現役訓練生の供血血漿からウイルスの輸血関連伝播が生じたことが判明したと報告である。

VFウイルスは脂質膜を有するRNAウイルスで、本剤の製造工程には、平成11年8月30日付医薬発第1047号に沿ったウイルス・ワロセスバリデーションによって検証された2つの異なるウイルス除去・不活化工程が含まれており、安全性は確保されていると考える。

今後の対応

日本赤十字社では、輸血感染症対策として黄熱等の生ワクチンを接種後4週間は献血不適としている。今後も引き続き、情報の収集に努める。

添付文書記載内容

赤+ケアルブミン20
赤+ケアルブミン25
赤+ケアルブミン20%静注 4g/20mL
赤+ケアルブミン20%静注 10g/50mL
赤+ケアルブミン25%静注 12.5g/50mL

血液を原料とすることに由来するウイルス感染症伝播等

JRC2010T-005

Centers for Disease Control and Prevention
Your Online Source for Credible Health Information

Morbidity and Mortality Weekly Report (*MMWR*)

Transfusion-Related Transmission of Yellow Fever Vaccine Virus --- California, 2009

Weekly
January 22, 2010 / 59(02);34-37

In the United States, yellow fever (YF) vaccination is recommended for travelers and active duty military members visiting endemic areas of sub-Saharan Africa and Central/South America (*1,2*). The American Red Cross recommends that recipients of YF vaccine defer blood product donation for 2 weeks because of the theoretical risk for transmission from a viremic donor (*3*). On April 10, 2009, a hospital blood bank supervisor learned that, on March 27, blood products had been collected from 89 U.S. active duty trainees who had received YF vaccine 4 days before donation. This report summarizes the subsequent investigation by the hospital and CDC to identify lapses in donor deferral and to determine whether transfusion-related transmission of YF vaccine virus occurred. The investigation found that a recent change in the timing of trainee vaccination had occurred and that vaccinees had not reported recent YF vaccination status at time of donation. Despite a prompt recall, six units of blood products were transfused into five patients. No clinical evidence or laboratory abnormalities consistent with a serious adverse reaction were identified in four recipients within the first month after transfusion; the fifth patient, who had prostate cancer and end-stage, transfusion-dependent, B-cell lymphoma, died while in hospice care. Three of the four surviving patients had evidence of serologic response to YF vaccine virus. This report provides evidence that transfusion-related transmission of YF vaccine virus can occur and underscores the need for careful screening and deferral of recently vaccinated blood donors.

On April 10, 2009, during a routine record review in connection with a subsequent blood drive, the blood bank supervisor learned of a breach in the deferral protocol for blood products collected from trainees. Further investigation revealed that the blood obtained in the previous drive was from trainees who had been vaccinated with YF vaccine 4 days before the drive. All of those blood products already had been processed and incorporated into the inventory at the hospital's blood bank. The blood bank supervisor reviewed blood bank records and identified 87 whole blood units and three apheresis platelet units obtained from the recently vaccinated trainees. Blood products that had been released for transfusion were tracked forward to identify the patients who had received the implicated blood products. Remaining unused blood products were identified and destroyed.

During April 20--30, investigators reviewed inpatient and outpatient records of patients who received the potentially infected blood products. A data collection tool was developed to capture demographic information, underlying medical conditions, blood product received, and information on previous YF vaccine doses. Because YF vaccine has been recognized to cause serious adverse events in persons who are immunocompromised or aged >60 years (*1*), information was collected on potential adverse events (e.g., fever, meningismus, mental status changes, elevated transaminases, or multisystem organ failure) that might have occurred during the 1 month after receipt of the blood products. All blood product recipients were notified in writing of the potential exposure to YF vaccine virus, and serum samples from the recipients were tested by enzyme-linked immunosorbent assay for immunoglobulin M (IgM) antibodies against YF virus (YFV). Samples testing positive for YFV-specific IgM antibodies were evaluated using the plaque reduction neutralization test, with a 90% cutoff value for neutralizing antibody titers against YFV (the standard evaluation at CDC for determining serologic response to YF vaccine virus). Additional testing for West Nile virus and St. Louis encephalitis virus IgM and IgG antibodies was performed using enzyme immunoassays to evaluate for possible cross-reactive flaviviral antibodies.

Blood Product Recipients

During March 31--April 9, five patients had received six blood products (three platelets, two fresh frozen plasmas, and one packed red cell unit) from six of the trainees. These six trainees had no previous history of vaccination or travel history consistent with exposure to wild-type YFV. In the month after the transfusion, one blood product recipient had died. The decedent was a man aged 82 years who was in hospice care for terminal prostate cancer and end-stage, transfusion-dependent, B-cell lymphoma. He died 20 days after receiving one of the implicated platelet units. No autopsy was performed, and no pre-mortem blood specimens were available for testing. The other four recipients of blood products had no documented laboratory abnormalities or symptoms attributable to YF vaccine (Table).

Residual blood products from the six transfusions had been discarded. Testing for pretransfusion serologic status of the blood product recipients could not be performed because banked sera were not available. However, serum samples drawn 26--37 days posttransfusion indicated that three of the four recipients had YFV-IgM antibodies confirmed by plaque reduction neutralization test. Testing for cross-reactive flaviviral infection by IgM and IgG antibodies was negative for all four recipients. Testing by reverse transcription--polymerase chain reaction or culture for the presence of YF vaccine virus in the surviving recipients was not performed because samples were obtained when viremia would no longer be expected if transfusion-related transmission had occurred. The patient without YFV-specific antibodies was a premature infant who received multiple aliquots of red blood cells from one donor. Of the three recipients demonstrating YF IgM antibodies, two had been previously vaccinated with YF vaccine at least 20 years earlier. A booster response was identified in these two previously vaccinated donor recipients by the presence of YFV-IgM antibodies and high neutralizing antibody titers (160 and 40,960, respectively).

Public Health Response

A review of records associated with the blood product donations confirmed that, in accordance with standard blood bank screening procedures, each trainee had been questioned regarding recent vaccinations on the day of donation. However, none reported having received YF vaccine 4 days earlier. To prevent a similar event in the future, personnel at the military training center now provides the blood bank with immunization records of all trainees at least 1 week before the blood drive, and just before donation, staff members ask each donor individually about his or her vaccination history.

Reported by

E Lederman, MD, T Warkentien, MD, M Bavaro, MD, J Arnold, MD, D DeRienzo, MD, US Navy. JE Staples, MD, M Fischer, MD, JJ Laven, OL Kosoy, RS Lanciotti, PhD, Div of Vector-Borne Infectious Diseases, National Center for Emerging and Zoonotic Infectious Diseases, CDC.

Editorial Note

This investigation documents, for the first time, serologic evidence for transmission of YF vaccine virus through infected blood products. Before this report, the risk for transmitting YF vaccine virus through blood products was only theoretical. From this investigation, various blood products, including irradiated platelets, appear capable of transmitting the YF vaccine virus. Although irradiation can minimize transfusion-associated graft-versus-host disease, the dose is inadequate to kill YF vaccine virus (A. Barrett, University of Texas Medical Branch, personal communication, 2009).

Of the four surviving blood product recipients, three had YFV-IgM and neutralizing antibodies. The one surviving recipient who did not have serologic evidence of exposures was a preterm infant. Two potential reasons for the lack of detectable levels of YFV-IgM antibodies in the preterm infant are the infant's immune system was not mature enough to mount an adequate immune response and lower levels of YF vaccine virus were present in red blood cells compared with other serum-containing products. Despite evidence of transmission of YF vaccine virus, no adverse events attributable to the transfused virus were identified in the blood recipients. In addition, these blood recipients were not ideal candidates for YF vaccination because of age or compromised immune status.

Persons receiving their first dose of YF vaccine often will develop a low-level viremia within 3--7 days after vaccination that persists for 1--3 days (*4*). As neutralizing antibody develops, viremia resolves. Neutralizing antibody develops in 90% of recipients within 10 days of vaccination and in 99% of recipients within 30

days (5). Immunity lasts for at least 10 years (1). Persons receiving subsequent doses typically do not develop viremia but might have an elevation in IgM antibodies if several years have passed since their last vaccination (6). YFV-IgM antibodies detected in the recipients might represent passive immunization (i.e., transfer of antibodies formed in the donor) rather than transmission of vaccine virus via blood product. However, this explanation is unlikely because all the donors were primary vaccine recipients, and they would be expected to have viremia with low or nonexistent levels of IgM antibodies at 4 days post-vaccination, when the blood donation occurred (7,8). Detection of YF vaccine virus in the original blood products or acute sera from recipients could have confirmed vaccine virus transmission, but samples were unavailable to perform such testing. Two of the three recipients with positive YFV-IgM antibody titers had been vaccinated previously with YF vaccine more than 20 years earlier likely had an anamnestic response to the vaccine virus in the blood products. This immunologic response is consistent with reports that YFV-IgM antibodies can reform after a booster dose of the vaccine, particularly with longer time between vaccinations (6,8).

Transfusion-related transmission of attenuated YF vaccine virus is preventable. Health-care providers should inform persons receiving live vaccines about the temporary deferral for blood donation. Providing additional checks and balances is especially important when blood product donors receive several vaccinations within a short period (e.g., in the case of active duty military personnel or travelers). If feasible, occupational health personnel at military training facilities should collaborate with the organizers of blood drives targeting military trainees to coordinate a minimum 2-week interval separating receipt of live vaccines and collection of blood products. All potential blood donors should be individually screened for a recent history of receipt of vaccines containing live virus during the month before donation, and temporary deferment should be based upon the expected post-vaccination period of viremia. Most temporary deferments due to receipt of live vaccines are 2 weeks; however, recipients of measles, mumps, and rubella vaccines and varicella vaccines should be deferred for 4 weeks because of the theoretical risk for prolonged viremia.

References

1. CDC. Yellow fever. In: CDC health information for international travel 2010. Atlanta, GA: US Department of Health and Human Services, Public Health Service; 2009. Available at http://wwwnc.cdc.gov/travel/yellowbook/2010/chapter-2/yellow-fever.aspx. Accessed January 14, 2010.
2. Grabenstein JD, Pittman PR, Greenwood JT, Engler RJM. Immunization to protect the US armed forces: heritage, current practice, and prospects. Epidemiol Rec 2006;28:3--26.
3. American Red Cross. Donating blood: eligibility requirements. Available at http://www.redcrossblood.org/donating-blood/eligibility-requirements/eligibility-criteria-alphabetical-listing . Accessed January 14, 2010.
4. Monath TP, McCarthy K, Bedford P, et al. Clinical proof of principle for ChimeriVax(tm): recombinant live, attenuated vaccines against flavivirus infections. Vaccine 2002;20:1004--18.
5. Barnett ED. Yellow fever: epidemiology and prevention. Clin Infect Dis 2007;44:850--6.
6. Reinhardt B, Jaspert R, Niedrig M, Kostner C, L'age-Stehr J. Development of viremia and humoral and cellular parameters of immune activation after vaccination with yellow fever virus strain 17D: a model of human flavivirus infection. J Med Virol 1998;56:159--67.
7. Monath TP. Neutralizing antibody responses in the major immunoglobulin classes to yellow fever 17D vaccination of humans. Am J Epidemiol 1971;93:122--9.
8. Bennevie-Nielsen V, Heron I, Monath TP, Calisher. Lymphocytic 2', 5' oligoadenylate synthetase activity increases prior to the appearance of neutralizing antibodies and immunoglobulin M and immunoglobulin G antibodies after primary and secondary immunization with yellow fever vaccine. Clin Diagn Lab Immunol 1995;2:302--6.

What is already known on this topic?

Blood donor centers temporarily defer donation from persons receiving live virus vaccines because of a theoretical risk for viral transmission to the blood product recipient.

What is added by this report?

Transfusion-related transmission of yellow fever vaccine virus is documented for the first time.

What are the implications for public health practice?

Blood donation centers should identify recipients of live virus vaccines to recommend the appropriate timeframe for deferral, which varies depending upon the timeframe for expected postvaccination viremia.

TABLE. Selected characteristics, clinical outcomes, and laboratory findings of five patients exposed to blood products from donors recently vaccinated with yellow fever vaccine --- California, 2009*

						Serologic evaluation	
Age	**Sex**	**Previous yellow fever vaccine (year)**	**Blood product received (quantity)**	**Underlying medical conditions**	**Symptoms and laboratory abnormalities†**	**Yellow fever virus IgM ELISA / PRNT§**	**No. of da post-transfusi**
Premature infant (24 wks estimated gestational age)¶	Female	No	Irradiated red blood cells (4 aliquots; 30 cc total)	Prematurity, intraventricular hemorrhage	None	Negative / Not done	37
6 yrs	Male	No	Irradiated platelets (1 unit)	Wilm's tumor (relapsed), recent chemotherapy	None	Positive / 160	36
66 yrs	Male	Yes (1964)	Platelets (1 unit)	Kidney/liver transplant (2005), diabetes, history of alcohol abuse	None	Positive / 160	33
58 yrs	Male	Yes (1975, 1986)	Fresh frozen plasma (2 units)	Chronic renal insufficiency, peritoneal and pulmonary tuberculosis, psoriasis (received infliximab >2 mos before)	None	Positive / 40,960	26
82 yrs	Male	Yes (1959, 1965)	Irradiated platelets (1 unit)	Diffuse large B cell lymphoma s/p chemotherapy and radiation treatment, prostate carcinoma	Deceased**	Premortem specimen not available for testing	---

* Based on electronic medical record review.

† In the 30 days after blood product transfusion (e.g., fever, rigors, headache, meningismus, paralysis, and mental status changes, and abnormalities in white blood cell count, transaminases, or cerebral spinal fluid [if clinically indicated]).

§ Immunoglobulin M enzyme-linked immunosorbent assay result and plaque reduction neutralization test titer.

¶ Received blood products during days 2, 4, 6, and 9 of life.

** Patient was discharged to inpatient hospice for underlying malignancy and died 20 days after receiving blood products. An autopsy was not performed.

別紙様式第２－１

医薬品　研究報告　調査報告書

番号：2

識別番号・報告回数			報告日	第一報入手日 平成 22 年 2 月 12 日	新医薬品等の区分 該当なし	機構処理欄
一般的名称	ﾃｸﾈﾁｳﾑ人血清ｱﾙﾌﾞﾐﾝ (99mTc)		研究報告の公表状況	CDC：MMWR/February 12, 2010/Vol.59/No.5	公表国 ブラジル	
販売名（企業名）	テクネアルブミンキット（富士ファイルムＲＩファーマ株式会社）					
研究報告の概要	要約：授乳を介した黄熱ワクチンウイルス感染： 2009 年 4 月にブラジルにおいて、母親が分娩後に黄熱ワクチンを接種し、乳児へ母乳を介して黄熱ワクチンウイルスが伝播したとの報告があった。 乳児は専ら母乳のみを摂取しており、抗痙攣薬による治療を要する髄膜脳炎が疑われる発作で生後 23 日で入院した。乳児の脳脊髄液からは 17DD 黄熱ワクチンウイルスが検出され、血清や脳脊髄液に黄熱特異的 IgM 抗体も認められた。調査の結果、乳児は母乳を介して黄熱ワクチンウイルスに感染したと特定された。 授乳中の女性に対する黄熱ワクチンの投与は、黄熱ウイルスへの暴露が避けられないあるいは延期できないという状況以外では避けるべきである。					使用上の注意記載状況・その他参考事項等 特になし
	報告企業の意見		今後の対応			
	黄熱ワクチンウイルスが母乳を介して感染伝播し、乳児が髄膜脳炎を発症したという報告。検査により確定された初めての報告であり、重大な感染症の新規感染経路に関する報告のため、感染症定期報告の対象と判断する。		本研究報告は、ヒト血液を原料とする血漿分画製剤とは直接関連しないことから、現時点で当該生物由来製品に関し、措置等を行う必要はないと判断する。			

MedDRA/J Version(13.0)

(9)

Transmission of Yellow Fever Vaccine Virus Through Breast-Feeding — Brazil, 2009

In April, 2009, the state health department of Rio Grande do Sul, Brazil, was notified by the Cachoeira do Sul municipal health department of a case of meningoencephalitis requiring hospitalization in an infant whose mother recently had received yellow fever vaccine during a postpartum visit. The Field Epidemiology Training Program of the Secretariat of Surveillance in Health of the Brazilian Ministry of Health assisted state and municipal health departments with an investigation. This report summarizes the results of that investigation, which determined that the infant acquired yellow fever vaccine virus through breast-feeding. The mother reported 2 days of headache, malaise, and low fever occurring 5 days after receipt of yellow fever vaccine. The infant, who was exclusively breast-fed, was hospitalized at age 23 days with seizures requiring continuous infusion of intravenous anticonvulsants. The infant received antimicrobial and antiviral treatment for meningoencephalitis. The presence of 17DD yellow fever virus was detected by reverse transcription–polymerase chain reaction (RT-PCR) in the infant's cerebrospinal fluid (CSF); yellow fever–specific immunoglobulin M (IgM) antibodies also were present in serum and CSF. The infant recovered completely, was discharged after 24 days of hospitalization, and has had normal neurodevelopment and growth through age 6 months. The findings in this report provide documentation that yellow fever vaccine virus can be transmitted via breast-feeding. Administration of yellow fever vaccine to breast-feeding women should be avoided except in situations where exposure to yellow fever viruses cannot be avoided or postponed.

On March 23, the mother, aged 22 years, delivered a healthy female infant at 39 weeks' gestational age by elective cesarean delivery. During that same month, a yellow fever epidemic had spread to a nonendemic area in Rio Grande do Sul state where the mother resided (*1*). On April 7, when the mother was 15 days postpartum, she visited her health-care provider to have the sutures removed from her cesarean incision. While in the provider's office, she received 17DD yellow fever vaccine. She had not been vaccinated for yellow fever previously. On April 12, 5 days after receiving the vaccine, she reported a headache, malaise, and low fever, which persisted for 2 days. The mother did not seek medical care for her symptoms.

On April 15, 2009, the mother's infant, aged 23 days, developed fever, and irritability and refused to nurse. The next day, the infant exhibited seizure-like activity and was admitted to the hospital for evaluation of possible meningoencephalitis. Upon admission, the infant experienced unilateral left upper extremity clonic convulsions of increasing frequency requiring intravenous diazepam (0.15 mg). Perioral cyanosis was noted and oxygen saturation measured by arterial blood gas was pO_2 60 (normal: pO_2 80–100). A chest radiograph showed no infiltrate. Peripheral white blood cell (WBC) count was 25,400/mm^3 (normal: 5,000–20,000 WBC/mm^3) and platelet count was 393,000/mm^3 (normal: ≥150,000 platelets/mm^3). Laboratory examination of CSF was unremarkable, with a WBC count of 1/mm^3 (normal: 0–5 WBC/mm^3), slight elevation of protein (67 mg/dL [normal: 15–45 mg/dL]), and decreased glucose concentration (37 mg/dL [normal: 42–78 mg/dL]). Gram stain of the CSF specimen revealed no bacteria. The infant received oxygen therapy, intravenous dipyrone (0.1 mL every 6 hours) and phenytoin (10 mg every 12 hours), and empiric treatment for bacterial infection with ampicillin and gentamicin. On April 18, empiric acyclovir treatment was added. No specimens for bacterial or fungal cultures were obtained. Other etiologies for meningoencephalitis were ruled out by testing of serum and CSF samples for dengue-specific IgM; viral culture for herpes simplex, cytomegalovirus, and varicella; and RT-PCR for enteroviruses, all of which were negative.

The infant alternated between periods of somnolence and irritability, without clinical improvement. On April 19, convulsions became more frequent (one episode every 10 minutes) and difficult to control, with persistent perioral cyanosis, resulting in transfer to the pediatric ICU for continuous infusion of anticonvulsants and monitoring of oxygen saturation. A second CSF examination showed a WBC count of 128/mm^3, a protein concentration of 106 mg/dL, and a glucose concentration of 24 mg/dL. Computerized tomography of the head demonstrated bilateral symmetrical areas of diffuse low density suggestive of inflammation consistent with encephalitis.

After the second CSF examination on April 19, the mother mentioned receiving yellow fever vaccine 8 days before the infant's onset of symptoms, and a serum and CSF sample from the infant were sent to the arbovirus reference laboratory at Adolfo Lutz Institute in São Paulo, Brazil, to test for the presence of 17DD yellow fever vaccine virus. Yellow fever–specific IgM antibodies were detected in serum and CSF. Yellow fever viral RNA was amplified by RT-PCR (*2,3*) from a CSF specimen collected on April 19; the nucleotide sequence of the amplified PCR product was identical to 17DD yellow fever vaccine virus. No breast milk or maternal serum was collected for yellow fever virus testing.

The infant recovered completely and was discharged from the hospital without sequelae on May 10, 2009. Follow-up of the infant showed normal neurodevelopment and growth through age 6 months. The Brazilian Committee on Vaccine-Associated Adverse Events classified the child's encephalitis as yellow fever vaccine–associated neurologic disease. To rule out the possibility that the infant had received yellow fever vaccine inadvertently, the investigators reviewed all procedures documented in the medical record performed between the infant's birth and onset of symptoms. The child had received intramuscular vitamin K and hepatitis B vaccine on the day of birth. Two other children born on the same day had received hepatitis B vaccine from the same lot of vaccine as the one registered in the child's vaccination record, and neither experienced similar symptoms.

Reported by

A Mallmann Couto, MD, M Ribeiro Salomão, MD, Hospital de Caridade de Cachoeira do Sul; MT Schermann, MD, R Mohrdieck, MD, Rio Grande do Sul State Health Dept, Porto Alegre; A Suzuki, Adolfo Lutz Institute, São Paulo; SM Deorti Carvalho, National Immunization Program, Secretariat of Surveillance in Health (SVS), Ministry of Health (MoH), Brasilia; DM de Assis, Brazilian Field Epidemiology Training Program (EPISUS) and Vector-borne Diseases and Anthropozoonoses Surveillance, SVS, MoH, Brasilia; W Navegantes Amâjo, DVM, EPISUS, SVS, MoH, Brasilia, and Gonçalo Moniz Institute, Oswaldo Cruz Foundation, MoH, Salvador; B Flannery, PhD, Pan American Health Organization, Brasilia, Brazil.

Editorial Note

This report describes the first laboratory-confirmed case of yellow fever vaccine–associated neurologic disease occurring in an infant secondary to the transmission of yellow fever vaccine virus through breast milk. The infant described in this report also is the youngest reported case of yellow fever vaccine–associated neurologic disease. The presence of yellow fever-specific IgM in CSF, and 17DD yellow fever vaccine viral RNA in the CSF of the infant indicates transmission and infection with yellow fever vaccine. Following primary vaccination, IgM antibodies generally appear 4–7 days after receipt of vaccine (*4*). Maternal IgM antibodies can be excreted in breast milk and the presence of serum IgM in the infant alone is not diagnostic of yellow fever virus infection. The detection of IgM antibodies in the infant's CSF indicates intrathecal antibody production in response to a nervous system infection because IgM does not normally cross the blood brain barrier (*5*).

Based on the mother's receipt of yellow fever vaccine on April 7, and onset of symptoms in the infant on April 15, the infant's infection likely occurred during the expected peak of viremia following vaccination. Neurologic adverse events, including encephalitis, have been described previously in association with yellow fever vaccination; children aged <6 months have the highest incidence of vaccine-associated neurologic events (*6*). However, only one previous episode of encephalitis, which was not confirmed as vaccine-associated, has been described in an infant exposed to yellow fever vaccine virus through breast-feeding (Public Health Agency of Canada, personal communications, 2009).

Yellow fever vaccine is a live, attenuated virus preparation made from various strains of the 17D yellow fever virus lineage. In Brazil, yellow fever vaccine from the 17DD strain is produced by Bio-Manguinhos, a public sector vaccine manufacturer of the Oswaldo Cruz Foundation of the Brazilian Ministry of Health. Yellow fever vaccine–associated neurologic disease (YEL-AND, formerly known as postvaccinal encephalitis) is reported to occur at a rate of 0.4 cases per 100,000 persons vaccinated in the U.S. population, with highest rates reported among persons aged ≥60 years (1.6 per 100,000) (*6*). However, the incidence among infants aged <6 months has been estimated as 0.5–4.0 cases per 1,000 infants vaccinated (*4*). For this reason, administration of 17D-derived yellow

What is already known on this topic?
Administration of yellow fever vaccine is contraindicated in children aged <6 months because of increased risk for vaccine-associated encephalitis; the Advisory Committee on Immunization Practices cautions against vaccinating breast-feeding women to avoid the potential risk for transmission of yellow fever vaccine virus to breast-feeding infants.

What is added by this report?
This report describes laboratory-confirmed, breast-feeding–associated transmission of 17DD yellow fever vaccine virus from a recently vaccinated mother; the affected infant developed postvaccinal encephalitis requiring hospitalization.

What are the implications for public health practice?
Health-care personnel should be aware that yellow fever vaccine virus can be transmitted through breast-feeding, and administration of yellow fever vaccine to breast-feeding women should be avoided except in situations where exposure to circulating yellow fever viruses cannot be avoided.

fever vaccines is contraindicated in infants aged <6 months (*4,7,8*).

Yellow fever virus, either wild-type or 17D, has not been reported to have been isolated from or detected in human breast milk. West Nile virus (WNV), another flavivirus, has been detected in milk from WNV-infected, lactating women (*9*), and one case of probable WNV transmission through breast-feeding has been reported (*10*). The actual risk for 17DD virus transmission through breast-feeding cannot be characterized because the number of breast-feeding women who have been vaccinated without negative sequelae in their infants is unknown. Based on the theoretical risk for yellow fever vaccine virus transmission through breast milk, the Advisory Committee on Immunization Practices recommends that yellow fever vaccination of nursing mothers be avoided, except when travel of nursing mothers to high-risk yellow fever–endemic areas cannot be avoided or postponed (*7*). Vaccine recommendations from the World Health Organization do not include considerations for breast-feeding mothers (*8*).

In Brazil, yellow fever vaccination is recommended for all residents of municipalities considered at risk for yellow fever transmission, and for travelers to at-risk areas (*1*). As a result of this investigation, the Brazilian Ministry of Health is revising its recommendations to caution against administration of yellow fever vaccine to breast-feeding women, except in situations where the risk for contracting yellow fever is unavoidable. Further studies on excretion of 17DD virus in breast milk of vaccinated, lactating women would help to define a risk period for viral transmission in cases where vaccination of nursing mothers is necessary.

Acknowledgments

The findings in this report are based, in part, on contributions from M Dallagnol, Municipal Dept of Health and Environment, Cachoeira do Sul; M Corrêa Lenz, M Assunta Bercini, MD, Rio Grande do Sul State Health Dept, Porto Alegre; R de Cássia Compagnoli Carmona, S Pires Curti, Adolfo Lutz Institute, São Paulo; and C Guedes Ramos, G Lima Nascimento, and MA Nunes Medeiros, Brazilian Field Epidemiology Training Program, Brasilia, Brazil.

References

1. Brazilian Ministry of Health. Emergências em Saúde Pública de Importância Nacional (ESPIN) de Febre Amarela Silvestre *em São Paulo e no Rio Grande do Sul e a Situação* Epidemiológica Atual no Brasil (2008/2009). [Portuguese] Available at http://portal.saude.gov.br/portal/arquivos/pdf/boletim_febre_amarela_09_12_09.pdf. Accessed February 9, 2010.
2. dos Santos CN, Post PR, Carvalho R, Ferreira, II, Rice CM, Galler R. Complete nucleotide sequence of yellow fever virus vaccine strains 17DD and 17D-213. Virus Res 1995;35:35–41.
3. Wang E, Weaver SC, Shope RE, Tesh RB, Watts DM, Barrett AD. Genetic variation in yellow fever virus: duplication in the 3' noncoding region of strains from Africa. Virology 1996;225:274–81.
4. Monath TP, Centeron MS, Teuwen DE. Yellow fever vaccine. In: Plotkin SA, Orenstein WA, Offit PA, eds. Vaccines. 5th ed. Philadelphia, PA: WB Saunders; 2008.
5. McMahon AW, Eidex RB, Marfin AA, et al. Neurologic *disease associated with 17D-204 yellow fever vaccination: a* report of 15 cases. Vaccine 2007;25:1727–34.
6. Khromava AY, Eidex RB, Weld LH, et al. Yellow fever vaccine: an updated assessment of advanced age as a risk factor for serious adverse events. Vaccine 2005;23:3256–63.
7. CDC. Yellow fever vaccine; recommendations of the Advisory Committee on Immunization Practices (ACIP). MMWR 2002;51(No. RR-17).
8. World Health Organization. Yellow fever vaccine: WHO position paper. Wkly Epidem Rec 2003;78:349–59.
9. Hinckley AF, O'Leary DR, Hayes EB. Transmission of West Nile virus through human breast milk seems to be rare. Pediatrics 2007;119:e666–71.
10. CDC. Possible West Nile virus transmission to an infant through breast-feeding—Michigan, 2002. MMWR 2002;51:877–8.

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 2010 年 3 月 3 日	新医薬品等の区分 該当なし	総合機構処理欄
一般的名称	別紙のとおり	研究報告の公表状況	Infect Genet Evol 9:1240–1247	公表国 フランス		
販売名（企業名）	別紙のとおり					

問題点：フランスの家禽と役場従業員に発生した非定型肺炎に関する調査から、クラミジアの新株の存在が示唆された。

研究報告の概要

フランスの家禽と役場従業員に発生した非定型肺炎に関する調査から、クラミジアの新たな株の存在が示唆された。と役場に家禽を供給した 10 農場における 25 群から得られた検体を用いて PCR 検査を行ったところ、同 25 群の内 14 群にクラミジア関連因子が認められた。同 14 群の内の 1 群の因子は Chlamydophila psittaci と同定されたものの、他の群の因子はこれまでに分類されていないものであった。未分類因子が認められた群の中の異なる 6 群の検体を用いた感染実験の結果、それらの 16S rRNA の遺伝子は非常に近い配列を有し、Chlamydophila 属に属することは明らかであるものの、同属の新たな株である可能性が示唆された。今のところ、これらの因子が人畜共通感染症の感染因子であるかは不明である。

報告企業の意見	今後の対応	使用上の注意記載状況・その他参考事項等
別紙のとおり	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。	記載なし

MedDRA/J ver.12.1

(10)

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人免疫グロブリン、⑤人免疫グロブリン、⑥人免疫グロブリン、⑦乾燥ペプシン処理人免疫グロブリン、⑧乾燥ペプシン処理人免疫グロブリン、⑨乾燥スルホ化人免疫グロブリン、⑩乾燥スルホ化人免疫グロブリン、⑪乾燥スルホ化人免疫グロブリン、⑫乾燥スルホ化人免疫グロブリン、⑬乾燥スルホ化人免疫グロブリン、⑭乾燥スルホ化人免疫グロブリン*、⑮乾燥濃縮人活性化プロテインC、⑯乾燥濃縮人血液凝固第Ⅷ因子、⑰乾燥濃縮人血液凝固第Ⅷ因子、⑱乾燥濃縮人血液凝固第Ⅸ因子、⑲乾燥濃縮人血液凝固第Ⅸ因子、⑳乾燥濃縮人血液凝固第Ⅸ因子、㉑乾燥抗破傷風人免疫グロブリン、㉒乾燥抗破傷風人免疫グロブリン、㉓乾燥濃縮人アンチトロンビンⅢ、㉔ヒスタミン加人免疫グロブリン製剤、㉕フィブリノゲン加第ⅩⅢ因子、㉖乾燥濃縮人アンチトロンビンⅢ、㉗抗 HBs 人免疫グロブリン、㉘抗 HBs 人免疫グロブリン、㉙トロンビン、㉚フィブリノゲン加第ⅩⅢ因子、㉛乾燥濃縮人アンチトロンビンⅢ、㉜乾燥濃縮人アンチトロンビンⅢ、㉝ヒスタミン加人免疫グロブリン製剤、㉞ヒスタミン加人免疫グロブリン製剤、㉟人血清アルブミン*、㊱人血清アルブミン*
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ガンマーグロブリン筋注 450mg/3mL「化血研」、⑥ガンマーグロブリン筋注 1500mg/10mL「化血研」、⑦献血静注グロブリン "化血研"、⑧献血グロブリン注射用 2500mg「化血研」、⑨献血ベニロン－Ⅰ静注用 500mg、⑩献血ベニロン－Ⅰ静注用 1000mg、⑪献血ベニロン－Ⅰ静注用 2500mg、⑫献血ベニロン－Ⅰ静注用 5000mg、⑬ベニロン*、⑭注射用アナクトC2、500 単位、⑮コンファクトF、⑯コンファクトF注射用 500、⑰コンファクトF注射用 1000、⑱ノバクトM、⑲ノバクトM注射用 250、⑳ノバクトM注射用 500、㉑ノバクトM注射用 1000、㉒テタノセーラ、㉓テタノセーラ筋注用 250 単位、㉔ヘパトセーラ、㉕ヘパトセーラ筋注 200 単位/mL、㉖トロンビン "化血研"、㉗ボルヒール、㉘ボルヒール組織接着用、㉙アンスロビンP、㉚アンスロビンP500 注射用、㉛ヒスタグロビン皮下注用、㉜アルブミン 20%化血研*、㉝アルブミン 5%化血研*、㉞静注グロブリン*、㉟アンスロビンP1500 注射用
報告企業の意見	クラミジア（Chlamydia）は 300nm 程度の大きさで、細胞内でのみ増殖する偏性細胞内寄生微生物であり、DNA と RNA を有し、2 分裂で増殖する。今回の報告は家禽と殺場従業員に発生した非定型肺炎に関する調査を機に、クラミジアの Chlamydophila 属における新株の可能性が示唆されたものであるが、それらが人畜共通感染症の感染因子であるかは不明である。 弊所で製造している全ての血漿分画製剤の製造工程には、約 0.2μm の無菌ろ過工程および、クラミジアよりも小さいウイルスの除去を目的としたウイルス除去膜ろ過工程が導入されているので、仮に製造原料にクラミジアが混入していたとしても、これらの工程により除去されるものと考えられる。更に、これまでに上記製剤によるクラミジア感染の報告例は無い。 以上の点から、上記製剤はクラミジア感染に対する安全性を確保していると考える。

*現在製造を行っていない

Infection, Genetics and Evolution 9 (2009) 1240–1247

Contents lists available at ScienceDirect

Infection, Genetics and Evolution

journal homepage: www.elsevier.com/locate/meegid

Isolation of a new chlamydial agent from infected domestic poultry coincided with cases of atypical pneumonia among slaughterhouse workers in France

Karine Laroucau [a,*], Fabien Vorimore [a], Rachid Aaziz [a], Angela Berndt [b], Evelyn Schubert [b], Konrad Sachse [b]

[a] Bacterial Zoonoses Unit, French Food Safety Agency (AFSSA), 23 avenue du Général de Gaulle, 94706 Maisons-Alfort, France
[b] Institute of Molecular Pathogenesis, Friedrich-Loeffler-Institut (Federal Research Institute for Animal Health), Jena, Germany

ARTICLE INFO

Article history:
Received 20 March 2009
Received in revised form 18 August 2009
Accepted 19 August 2009
Available online 26 August 2009

Keywords:
Chlamydophila spp.
New agent
Domestic poultry
Real-time PCR
Genotyping
DNA sequence analysis

ABSTRACT

Three cases of atypical pneumonia in individuals working at a poultry slaughterhouse prompted an epidemiological survey in 10 poultry farms that had supplied birds. Using a *Chlamydiaceae*-specific real-time PCR assay, chlamydial agents were detected in 14 of 25 investigated flocks. Rather unexpectedly, *Chlamydophila psittaci* was identified only in one of the positive flocks, whereas ArrayTube DNA microarray testing indicated the presence of a new, so far unclassified member of the genus *Chlamydophila*.

For further characterization of the agent involved, positive cloacal swabs were used to inoculate embryonated chicken eggs and isolates were obtained from 6 different flocks. Sequencing of 16S rRNA genes revealed nearly identical sequences of all samples. Alignment with representative sequences of *Chlamydiaceae* showed the separate position of the present strains outside the currently recognized species of *Chlamydophila*, but clearly within this genus. In contrast, partial *ompA* gene sequences displayed considerable diversity among the isolates, which had already been observed in restriction enzyme analysis of *ompA* PCR products. These data suggest that each farm had been infected with a different strain of this new chlamydial agent, the zoonotic potential and the exact taxonomic status of which have yet to be defined.

1. Introduction

Chlamydial infections leading to outbreaks of avian chlamydiosis in domestic, companion and wild birds are regularly reported from all parts of the world. As their general importance is based on two aspects, i.e. economic losses to the bird owners and potential zoonotic transmission to humans, control measures are obligatory in a number of European countries, where specific state legislation is in force. The most prominent chlamydial agent in *Aves* is *Chlamydophila* (*C.*) *psittaci*, which was shown to occur in as many as 465 bird species (Kaleta and Taday, 2003). Following the recent revision of chlamydial taxonomy (Everett et al., 1999), this obligate intracellular bacterium now predominantly comprises avian serovars. The family *Chlamydiaceae* with its two genera *Chlamydia* and *Chlamydophila* currently combines a total of nine species, i.e. *Chlamydia trachomatis*, *Chlamydia suis* and *Chlamydia muridarum*, as well as *C. pneumoniae*, *C. abortus*, *C. caviae*, *C. felis*, *C. pecorum*, and *C. psittaci*, respectively.

The importance of *C. psittaci* as the causative agent of psittacosis or avian chlamydiosis in psittacine birds and domestic fowl has been known for decades. A number of recent reports have confirmed that its zoonotic potential remains significant in the face of regularly occurring outbreaks of disease in domestic fowl (Vanrompay et al., 1995; Gaede et al., 2008; Laroucau et al., 2009). In addition, infections can take a subclinical and/or chronic course (Harkinezhad et al., 2009). However, occasional detections of *C. abortus* (Herrmann et al., 2000; Pantchev et al., 2008) and so far non-classified chlamydial agents (Gaede et al., 2008), as well as genetic evidence on intermediate strains between *C. psittaci* and *C. abortus* (Van Loock et al., 2003) suggest that the spectrum of *Chlamydiaceae* spp. encountered in birds is not confined to a single species.

In this context, it should be noted that laboratory diagnosis of infections involving chlamydiae has undergone a remarkable methodological change in the past two decades (Sachse et al., 2009). While only a few specialized laboratories are still conducting routine isolation of chlamydiae using cell culture or embryonated eggs, DNA-based detection methods have become widely accepted. This implies that specific PCR tests for individual chlamydial species and/or pan-*Chlamydiaceae* assays are conducted, which are capable of detecting even small amounts of a known agent within a working day. However, new and hitherto

Abbreviations: MOMP, major outer membrane protein; RFLP, restriction fragment length polymorphism; rtPCR, real-time PCR.

* Corresponding author. Tel.: +33 1 49 77 13 00; fax: +33 1 49 77 13 44.
E-mail address: k.laroucau@afssa.fr (K. Laroucau).

1567-1348/$ – see front matter © 2009 Elsevier B.V. All rights reserved.
doi:10.1016/j.meegid.2009.08.005

non-classified taxa will often be overlooked by this approach, so that the inclusion of a highly parallel screening assay, e.g. a DNA microarray test, is advisable.

Following the occurrence of symptoms of atypical pneumonia in a group of poultry slaughterhouse workers in France early in 2008, a diagnostic investigation in the slaughterhouse and the flocks of the suppliers was undertaken. Initial tests suggested involvement of chlamydiae, so that diagnostic examinations focused on *Chlamydiaceae* spp. In the present paper, we report the results of this study, which led to the identification of so far non-classified avian chlamydial agents.

2. Materials and methods

2.1. History of human infections

In the period from April 25 to May 30 in 2008, three individuals presented to their physicians with atypical pneumonia. Case 1, a 49-year-old woman, had fever and flu-like symptoms and was administered Naxy. Case 2 was a 25-year-old woman suffering from thoracic pain and fever. She had to be hospitalized and was treated with Clamoxyl and Rulide. Case 3, a man aged 62, had fever and complained about weariness. He was administered Zeclar. No diagnostic data from microbiology and serology are available.

2.2. Animal samples

Cloacal swabs were collected from poultry flocks received for slaughter and from birds belonging to the slaughterhouse owner. In each sampled flock, 10 animals were examined. One panel of swabs was stored in 1 ml of conservation buffer SPG (Spencer and Johnson, 1983) at −80 °C until inoculated into chicken eggs, whereas the other panel was stored dry at −80 °C until subjected to DNA extraction. Data on age, breed and flock of the examined birds are contained in Table 1.

2.3. DNA extraction

Dry cloacal swabs were subjected to DNA extraction using the QIAamp DNA Mini Kit (QIAGEN, Courtaboeuf, France) following the buccal swab protocol. For DNA extraction from aliquots of chlamydial cell culture and vitellus membranes, the tissue protocol of the same kit was used. Finally, DNA was eluted with 150 μl of AE buffer and stored at −20 °C until examination.

2.4. Direct detection of chlamydiae by real-time PCR

A *Chlamydiaceae*-specific real-time PCR targeting the 23S rRNA gene (23S-rtPCR) was used in this study (Ehricht et al., 2006). The protocol includes primers Ch23S-F (5′-CTGAAACCAGTAGCTTA-TAAGCGGT-3′), Ch23S-R (5′-ACCTCGCCGTTTAACTTAACTCC-3′), and probe Ch23S-p (FAM-5′-CTCATCATGCAAAAGGCACGCCG-3′-TAMRA). Each reaction mix contained 2 μl sample DNA template, 10 μl of Universal Mastermix 2× (Applied Biosystems, Courtaboeuf, France), 0.5 μl of each primer (25 μM), 2 μl of the probe (1 μM), and 5 μl deionized water. The temperature–time profile was 95 °C for 10 min, 45 cycles of 95 °C for 15 s and 60 °C for 60 s.

The *ompA*-based real-time PCR assay specific for *C. psittaci* was conducted as recently described (Pantchev et al., 2008).

2.5. Culture on embryonated chicken eggs

For culture, suspensions of cloacal swabs stored in conservation buffer at −80 °C were thawed, transferred into sterile Eppendorf tubes and centrifuged at 10,000 rpm for 5 min. Supernatant was transferred into a new sterile tube. Antibiotic solution containing 0.1 mg of vancomycin, 0.1 mg of streptomycin, 0.1 mg of kanamycin and 100 U of nystatin was added to the supernatant and the pellet suspensions, which were then incubated at 37 °C for 2 h before inoculation.

Yolk sacs of 7-day-old embryonated eggs were inoculated with 0.2 ml per egg, and five eggs per sample. For each set of inoculation, three eggs were inoculated with *C. psittaci* strain Loth as positive control, and three other eggs were kept separately as non-infected controls. Eggs were incubated at 38 °C and observed daily.

Vitellus membranes were collected and analyzed using a MIF test, i.e. a direct *immunofluorescence assay* (Chlamydia direct IF, BioMérieux, Marcy l'Etoile, France). One of the two monoclonal antibodies contained in this kit allowed the identification of *Chlamydiaceae* spp.

Table 1
Characteristics of investigated poultry flocks and results of diagnostic testing.

Flock ID	Breeder no.	Flock effective	Breed	Age at time of sampling (weeks)	23S-rtPCR *Chlamydiaceae*		*ompA*-rtPCR *C. psittaci*	Cell culture isolation
					No. of +ve samples	Mean Ct	No. of +ve samples	
Slaughterhouse								
08-1274/2	Slaughterhouse	500	Chicken	4	1/10 (10%)	38.6	0/1	
08-1274/1	breeder	500	Chicken	8	0/10	–	–	
08-1274/5		500	Chicken	12	0/10	–	–	
08-1274/4		500	Chicken	16	4/10 (40%)	33.0	0/4	
08-1274/3		500	Chicken	20	10/10 (100%)	28.8	0/10	+
08-1274/6		100	Guinea fowl	7	0/10	–	–	
08-1274/8		120	Guinea fowl	13	0/10	–	–	
08-1274/7		120	Guinea fowl	16	0/10	–	–	
Customers								
08-1274/9	Breeder 1	320	Chicken	20	3/10 (30%)	34.6	0/3	
08-1274/10		–	Guinea fowl	20	0/10	–	–	
08-1274/17		–	Duck	–	1/5 (20%)	35.1	1/1	+
08-1274/11	Breeder 2	600	Chicken	15	0/10	–	–	
08-1274/12		–	Guinea fowl	15	1/8 (12.5%)	40.1	0/1	
08-1274/13	Breeder 3[a]	600	Chicken	–	9/9 (100%)	26.3	0/9	+
08-1274/23		600	Chicken	12	10/10 (100%)	27.2	0/10	+
08-1274/24		600	Chicken	4	3/10 (30%)	39.0	0/3	
08-1274/14	Breeder 4	–	Chicken	–	0/10	–	–	
08-1274/15		–	Guinea fowl	–	0/8	–	–	
08-1274/16	Breeder 5[a]	600	Chicken	8	1/10 (10%)	38.8	0/1	
08-1274/19		600	Chicken	18	10/10 (100%)	27.0	0/10	+
08-1274/18	Breeder 6[a]	600	Chicken	18	0/10	–	–	
08-1274/20	Breeder 7[a]	600	Chicken	12	1/10 (10%)	38.9	0/1	
08-1274/21		600	Chicken	17	10/10 (100%)	26.6	0/10	+
08-1274/22	Breeder 8[a]	600	Chicken	16	9/10 (90%)	31.8	0/9	+
08-1274/25	Reproducer	407	Chicken	43	0/10	–	–	

[a] Breeders linked to the same supplier of 1-day-old chickens, i.e. 08-1274/25.

Table 2
Primers used for sequencing.

Targeted sequence	Primer name	Sequence[a] (5′-3′)	PCR product size	References
16S rRNA	16S1	CGGATCCTGAGAATTTGATC	1200 bp	Pudjatmoko et al. (1997) Thomas et al. (2006)
	rp2	CTACCTTGTTACGACTTCAT		
ompA	191CHOMP	GCIYTTTCGGGARTGYGGITGYGCIAC	600 bp	Sachse and Hotzel (2003)
	CHOMP371	TTAGAAICKGAATTGIGCRTTIAYGTGIGCIGC		

[a] Degenerate nucleotides: K = G, T; M = A, C; W = A; T; Y = C, T; I = Inosine.

2.6. Cell culture

Chlamydial isolates were grown in Buffalo Green Monkey (BGM) kidney cells as previously described (Sachse et al., 2003). Three days after inoculation, coverslips were fixed with methanol, and the monolayer was stained using the IMAGEN™ Chlamydia kit (DAKO Ltd., Cambridgeshire, UK). For confocal laser scanning microscopy using a TCS-SP2 confocal microscope (Leica, Bensheim, Germany), the coverslips were stained with Evans Blue, FITC-labeled anti-Chlamydia antibody (Oxoid, Cambridgeshire, UK) and DAPI (Sigma, Taufkirchen, Germany).

2.7. DNA-based characterization

2.7.1. DNA microarray testing

The ArrayTube™ (AT) assay was used as described (Borel et al., 2008) to check samples for the presence of DNA from any of the currently accepted nine species of *Chlamydia* and *Chlamydophila*, as well as *Waddlia chondrophila* and *Simkania negevensis*. Briefly, DNA template was amplified and biotin labeled in 40 cycles of 94 °C/30 s, 55 °C/30 s, and 72 °C/30 s, using primers U23F-19 (5′-ATTGAMAGGCGAWGAAGGA-3′) and 23R-22 (5′-biotin-GCYTAC-TAAGATGTTTCAGTTC-3′). Hybridization was conducted in the AT vessel at 58 °C for 1 h. Subsequently, the array was washed, and hybridization patterns were visualized using streptavidin-conjugated peroxidase-catalyzed precipitation and processed using the ATR-01 reader (Clondiag Chip Technologies, Jena, Germany) and the Iconoclust 2.3 program (Clondiag).

2.7.2. Multiple locus VNTR analysis

Samples were examined using the recently published MLVA procedure (Laroucau et al., 2008a).

2.7.3. Amplifications for DNA sequencing

Primers used for the partial amplification of *ompA* and 16S rRNA genes are listed in Table 2. PCR was performed in a total volume of 50 μl containing 4 μl of DNA template, 5 μl of 10× PCR reaction buffer, 2 U of Hot start *Taq* DNA polymerase (Qiagen), 400 μM of each deoxynucleotide triphosphate, and 0.5 μM of each flanking primer. The temperature–time profile for amplification of the 16S rRNA gene was 95 °C for 10 min, 4 cycles of 95 °C for 1 min, 40 °C for 1 min, 72 °C for 2 min and 36 cycles of 95 °C for 1 min, 50 °C for 1 min, 72 °C for 2 min. The final extension step was at 72 °C for 10 min.

2.7.4. Genotyping using restriction fragment length polymorphism (RFLP) of ompA amplicons

Aliquots of 10 μl of PCR product (primers 191CHOMP/CHOMP371, from Sachse and Hotzel, 2003) were subjected to overnight restriction enzyme digestion using 1 U of *AluI* (Euromedex, Souffelweyersheim, France) in a total volume of 15 μl at 37 °C. The digestion products were then loaded onto a 4% gel (50% Nusieve–50% Metaphore) containing ethidium bromide and analyzed using a UV transilluminator.

2.7.5. DNA sequencing

PCR-amplified segments of *ompA* and 16S rRNA genes were purified using the QIAquick PCR Purification Kit (Qiagen). DNA sequencing was done at MWG Biotech France (Roissy, France). Nucleotide sequences have been deposited under GenBank no. GQ398026 to GQ398031 for 16S rRNA sequences and under GenBank no. GQ398032 to GQ398038 for *ompA* sequences.

2.7.6. Sequence analysis

All 16S rRNA and *ompA* gene sequences determined in this study were combined in an alignment with previously published sequences that were relevant for phylogenetic and epidemiological considerations. Accession numbers of these sequences are listed in Table 3. Sequence data were analyzed using the Bionumerics software package version 4.6 (Applied-Maths, Saint-Martens-Latem, Belgium) as a character dataset. Cluster analysis was conducted using the categorical parameter and the UPGMA coefficient.

3. Results

3.1. Examination of samples from infected flocks

In preliminary examinations following diagnosis of atypical pneumonia in humans, two working-surface area samples from the slaughterhouse where those workers were employed, 20 swabs collected from chickens and guinea fowl belonging to the flock of the slaughterhouse owner and two fecal samples from the same flock were tested by real-time PCR. The results given in Table 4 revealed the presence of *Chlamydiaceae* in the birds as well as in the slaughterhouse itself. Quite surprisingly, none of the samples was positive for *C. psittaci*.

Subsequently, samples were collected from 16 poultry flocks, all of which were regular suppliers to this slaughterhouse. The prevailing bird species were chickens of the "Barbezieux" and "Coucou" breeds, but guinea fowl (three flocks) and ducks (one flock) were also included. In addition, samples were taken from 8 flocks belonging to the slaughterhouse owner and the flock of the egg supplier of five breeders raising "Barbezieux" chickens (Breeders no. 3, 5, 6, 7 and 8). Essential data and testing results are given in Table 1. In real-time PCR examinations, *Chlamydiaceae* were detected in 14 flocks. Comparison of Ct values indicated different

Table 3
BLAST analysis of partial *ompA* sequences (about 480 nt) of samples from flocks 08-1274/3, 08-1274/9, 08-1274/13, 08-1274/19, 08-1274/21 and 08-1274/22.

Origin of strain	*ompA* partial fragment size (nt)	GenBank acc. no. of partial *ompA* sequence	Highest similarity to (GenBank acc. no.)	Total BLAST score	Query coverage	E value	Max identity
08-1274/3	480 bp	GQ398033	*Chlamydophila* sp. 6617-T5 (EU019096)	479	60%	6.00E−132	96%
			Chlamydophila sp. 6620-T4 (EU019095)	479	60%	6.00E−132	96%
			Chlamydophila sp. 6688-T2 (EU019094)	440	62%	3.00E−120	93%
08-1274/9 (swab)	489 bp	GQ398034	*Chlamydophila* sp. 6617-T5 (EU019096)	695	77%	0	99%
			Chlamydophila sp. 6620-T4 (EU019095)	686	76%	0	99%
			Chlamydophila sp. 6688-T2 (EU019094)	455	60%	1.00E−124	94%
			Chlamydophila sp. PEENT (U82955)	324	59%	3.00E−85	86%
08-1274/13 & 08-1274/23	480 bp	GQ398035, GQ398032	*Chlamydophila* sp. 6617-T5 (EU019096)	484	60%	1.00E−133	96%
			Chlamydophila sp. 6620-T4 (EU019095)	484	60%	1.00E−133	96%
			Chlamydophila sp. 6688-T2 (EU019094)	457	62%	3.00E−125	94%
08-1274/19	486 bp	GQ398036	*Chlamydophila felis* MOMP gene for major outer membrane protein (X61096)	182	30%	2.00E−42	89%
08-1274/21	489 bp	GQ398037	*Chlamydophila* sp. 6617-T5 (EU019096)	363	76%	6.00E−97	84%
			Chlamydophila sp. 6620-T4 (EU019095)	359	76%	8.00E−96	84%
			Chlamydophila sp. PEENT (U82955)	353	61%	4.00E−94	88%
			Chlamydophila sp. 6688-T2 (EU019094)	348	75%	2.00E−92	84%
08-1274/22	483 bp	GQ398038	*Chlamydophila* sp. 6688-T2 (EU019094)	320	74%	4.00E−84	82%
			Chlamydophila felis MOMP gene for major outer membrane protein (X61096)	204	72%	4.00E−49	77%

Table 4
Summary of preliminary diagnostic testing.

1st investigation	No. of analyzed samples	23S-rtPCR *Chlamydiaceae* No. of positive samples	*ompA*-rtPCR *C. psittaci* no. of positive samples
In the slaughterhouse	2	1/2	0/1
In the personal flock of the owner (chickens and guineafowl)			
Cloacal swabs	20	5/20	0/5
Fecal samples	2	1/2	0/1

levels of chlamydial excretion, with birds from flocks no. 3, 13, 19, 21, 22, and 23 being identified as high excretors and with almost 100% of animals testing positive. Notably, flock 08-1274/25, which was linked with flocks 08-1274/13, 08-1274/16, 08-1274/18, 08-1274/19, 08-1274/20, 08-1274/21, 08-1274/22, 08-1274/23 and 08-1274/24 for being their exclusive supplier of 1-day-old chicks, proved negative when tested.

Again, *C. psittaci* was not the predominant chlamydial agent, since only a single sample (from flock no. 08-1274/17, the only investigated duck flock) was positive in *C. psittaci*-specific real-time PCR and, subsequently, also in MLVA. The *C. psittaci*-specific MLVA genotype of the duck sample was 2:3:2:0:6:3:0:4 using primers ChlaPsi_280, ChlaPsi_480, ChlaPsi_605, ChlaPsi_810, ChlaPsi_222, ChlaPsi_281, ChlaPsi_929 and ChlaPsi_1778, respectively (data not shown).

3.2. Direct genotyping of clinical samples

While PCR using the classical *ompA* primers CTU/CTL (Denamur et al., 1991) failed to produce amplicons from positive non-*C. psittaci* samples (data not shown), the use of degenerate primers 191CHOMP/CHOMP371 enabled further characterization by RFLP. DNA extracts from real-time PCR-positive (Ct < 34) cloacal swabs were subjected to PCR and digested with *AluI*. As shown in Fig. 1, all restriction patterns within a flock were identical, but clearly differed among flocks 08-1274/3, 08-1274/9, 08-1274/13, 08-1274/21, 08-1274/22, and 08-1274/19. As the only exception, patterns from flocks 08-1274/3 and 08-1274/4 were identical. These 2 flocks belonged to the same breeder (i.e. the owner of the slaughterhouse, see Table 1). The same was observed for flocks 08-1274/13 and 08-1274/23, which also belonged to the same breeder (Breeder no. 3).

3.3. DNA-based characterization of isolated strains

In cell culture trials, duplicates of the PCR-positive dry swabs, which had been stored in SPG medium, were inoculated into embryonated chicken eggs. Isolates were successfully cultured from flocks 08-1274/3, 08-1274/13, 08-1274/19, 08-1274/21, 08-1274/22 and 08-1274/23 (Table 1). The same strains also grew well

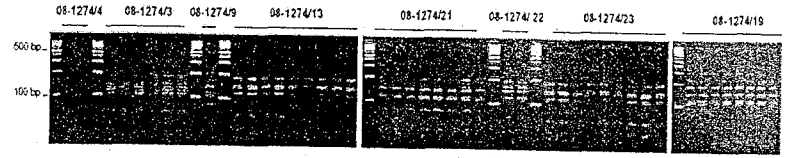


Fig. 1. Genotyping of cloacal swab samples by RFLP. *AluI* enzyme restriction profiles of partial *ompA* PCR products from clinical samples of 8 flocks. The following samples from the respective flocks were examined: 08-1274/4 (2 birds), 08-1274/3 (6 birds), 08-1274/9 (1 bird), 08-1274/13 (9 birds), 08-1274/21 (8 birds), 08-1274/22 (2 birds), 08-1274/23 (9 birds) and 08-1274/19 (8 birds). DNA size marker (GeneRuler™ 100 bp, Euromedex, France) was loaded between each flock. Fragment sizes (in bp) are given on the left-hand margin.

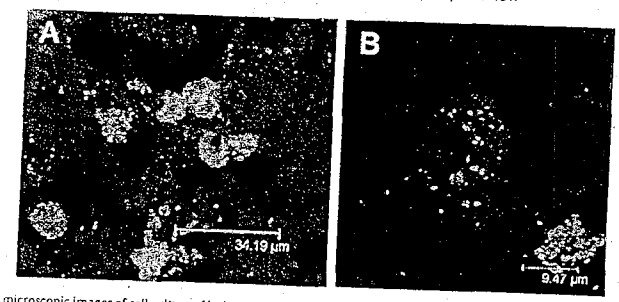


Fig. 2. Confocal laser scanning microscopic images of cell culture of isolate 08-1274/3. The chlamydial agent was grown in BGM cells, fixed with methanol on coverslips, and the monolayer was stained using Evans Blue for BGM cells (red color), FITC-labeled anti-Chlamydia antibody for chlamydial bodies (green, A) and DAPI for BGM cell nuclei (blue, A). Yellow: co-localization of chlamydial inclusions and cellular Evans Blue (A). FITC-labeled anti-Chlamydia antibody for chlamydial bodies (B). Scale bars are given on each image. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

in cell culture of BGM cells, where intracellular inclusions reminiscent of other chlamydial agents were observed (Fig. 2). DNA extracts from these cultures were examined by DNA microarray analysis using the AT test. Fig. 3 shows a typical hybridization pattern, where only the genus-specific probes generated specific positive signals, whereas species-specific signals were absent. These results indicated that DNA of any of the 9 established species of *Chlamydiaceae* was not present in the samples.

To establish the identity of the strains encountered in the poultry flocks, partial sequencing of the *ompA* gene was conducted for 6 isolates (from flocks 08-1274/3, 08-1274/13, 08-1274/19, 08-1274/21 08-1274/22 and 08-1274/23) and one swab sample from flock 08-1274/9. BLAST analysis shown in Table 3 revealed that *ompA* sequences of samples from flocks 08-1274/3, 08-1274/9 and 08-1274/13 exhibited the highest degree of similarity to a group of sequences from an outbreak of psittacosis in Germany (EU019094–EU019096), which had been tentatively classified as *Chlamydophila* spp. because they could not be assigned to any of the currently defined species (Gaede et al., 2008). Furthermore, *ompA* sequences of isolates from flocks 08-1274/9 and 08-1274/21 were found to have moderate similarity to another non-classified strain of *Chlamydophila* spp. (U82955) isolated from a peacock. The isolates from flocks 08-1274/19 and 08-1274/22 displayed moderate similarity to the *C. felis* ompA sequence and to one of the group of sequences from an outbreak of psittacosis in Germany, respectively. Sequences from isolates 08-1274/13 and 08-1274/23 (same breeder) were identical.

To explore the genetic relatedness of the strains from the present study, all sequences mentioned in Table 3 were aligned

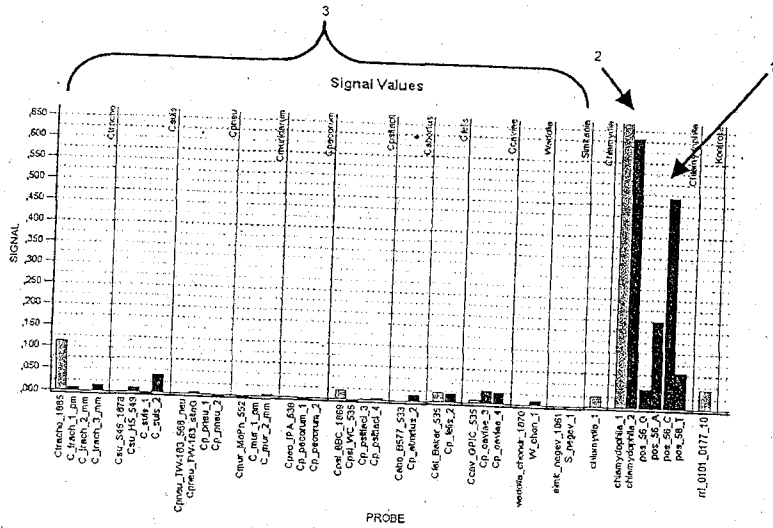


Fig. 3. Hybridization pattern obtained from examination of isolate 08-1274/3 using the ArrayTube DNA microarray assay. Barplot of hybridization signals: 1 consensus probe (family *Chlamydiaceae*), 2 genus-specific probes (*Chlamydophila*), 3 probes specific for the currently defined nine species of *Chlamydiaceae*.

with *ompA* sequences of strains representing the established species of *Chlamydia* and *Chlamydophila*. The dendrogram shown in Supplement 1 indicates that the strains described here form a separate cluster situated at the margin of the genus *Chlamydophila*.

As the *ompA* gene generally is distinguished by high intra-species diversity among chlamydiae, analysis of the more conserved 16S rRNA gene sequences was conducted to obtain alternative information on the identity and characterize the

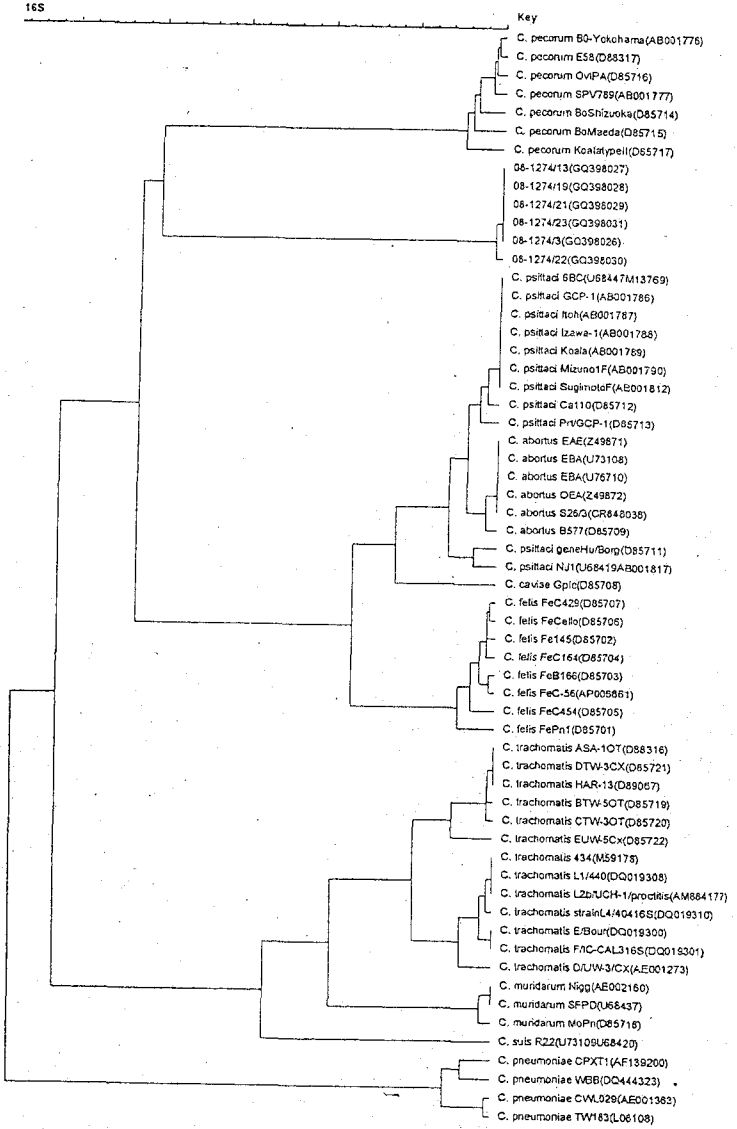


Fig. 4. Dendrogram for *Chlamydiaceae* based on a partial sequence analysis 16S rRNA genes. The tree was constructed by the neighbour-joining method from phylogenetic distances calculated by UPGMA method. Horizontal distances correspond to genetic distances, vertical distances are arbitrary.

taxonomic position of the present isolates. Segments of 1436 nt representing nearly the entire gene were sequenced from samples from the five strains and one clinical sample mentioned in Table 4. All sequences obtained were identical except for a single point mutation in 08-1274/22. Alignment with representative sequences of *Chlamydiaceae* confirmed the separate position of the present strains outside the existing species of *Chlamydophila*, but clearly within the genus. The corresponding dendrogram is shown in Fig. 4. Sequence similarity values given in Supplement 2 also clearly show the distinct genetic position of the present strains.

4. Discussion

So far, very few studies on avian chlamydiosis in chickens have been conducted in France and elsewhere. Veterinary investigations are usually undertaken when transmission to humans is suspected. To obtain more information, an epidemiological study on psittacosis involving 15 French administrative units is currently underway (http://www.invs.sante.fr/surveillance/psittacose/default.htm), whose aim is the determination of the incidence of hospitalized human cases, as well as the frequency of grouped cases, and risk assessment for exposed individuals. Additionally, the analysis of strains isolated from humans and animals and the description of breeding characteristics and working conditions should improve the knowledge on risk factors for animal-to-human transmission. Up to now, most of the recently confirmed cases of *C. psittaci* infection have been associated with ducks or exotic birds (Laroucau et al., 2008b, and unpublished data).

The present survey was prompted by the occurrence of hitherto unexplained atypical pneumonia in three French slaughterhouse workers in 2008. This poultry slaughterhouse had originally not been included in the national study mentioned above, but a veterinary survey was started as symptoms of the workers were reminiscent of psittacosis, without any microbiological confirmation.

Although no clinical signs were seen in the birds, diagnostic testing revealed the presence of *Chlamydiaceae* in most of the poultry flocks investigated, and some of the flocks were identified as high excretors. The levels of excretion were similar to those previously observed in *C. psittaci*-infected duck flocks, some of them associated with human infections (Laroucau et al., 2009). Rather unexpectedly, only one out of 73 *Chlamydiaceae*-positive samples of the present panel proved positive in *C. psittaci*-specific real-time PCR. When genotyping of the chlamydial strains involved was attempted, *C. psittaci*-specific VNTR primers failed to generate patterns characteristic for this species (except for the positive sample). Subsequently, the AT test revealed aberrant hybridization patterns, i.e. signals of the genus-specific probes for *Chlamydophila*, but the absence of grouped species-specific signals (Fig. 3). This combined evidence suggested that we were probably dealing with a novel chlamydial agent.

Analysis by RFLP of *ompA* gene segments directly amplified from the most high-titer real-time PCR-positive samples indicated the presence of a single strain within each investigated flock, but also revealed that the strains were different from each other. Thus, 6 isolates were obtained from 6 different flocks raising "barbezieux" or "cou nu" breeds. Two of them, which were isolated at the same farm, proved identical based on their partial *ompA* gene sequences (flocks 08-1274/13 and 08-1274/23). Partial sequencing of the 16S rRNA and *ompA* genes revealed that, while 16S rRNA gene sequences were highly similar among the isolates, the *ompA* sequences were distinguished by high inter-strain heterogeneity. This confirms observations by Everett et al. (1999), who pointed out that rRNA genes were subjected to evolutionary pressure to a far lesser extent than genes encoding outer membrane proteins, such as *ompA*. The same authors recommended that, in order to be classified as a member of *Chlamydiaceae*, a taxon should have less than 10% 16S rRNA gene diversity to any other member of the family. This condition is fulfilled for the isolates described in this study. Furthermore, comparison of the 16S rRNA sequences with those of the established species of *Chlamydiaceae* showed that the present avian strains formed a separate cluster within the genus *Chlamydophila* (Fig. 4). While the evidence gathered so far indicates that the 6 isolates belong to a new species, the authors are aware that more DNA sequence data, as well as morphological and other phenotypic data, are required to justify the definition of a new taxon. To address the epidemiological importance, we will further pursue the question whether these new microorganisms are occurring in other regions and countries. In any case, the present idea of taxonomic classification is still preliminary.

The present investigations were conducted in a limited geographical area, which involved, among others, a small poultry production unit dedicated to the barbezieux chicken breed. This chain involved a unique breeder (parental) flock which supplied 5 other breeders (Breeders no. 3, 5, 6, 7 and 8) dealing with fattening. The birds were all slaughtered in the slaughterhouse concerned. The first surprise was that one of the five breeders (Breeder no. 6) was not affected by these new *Chlamydophila* bacteria (Table 1). Six months later, follow-up sampling was done in another flock of this breeder, and, again, no *Chlamydiaceae* were detected in 10 animals randomly selected and examined 5 times in 16 weeks (at 3, 6, 8, 12 and 16 weeks of age, data not shown). Another surprise was the finding that the four farms having infected flocks harbored different strains, as shown by partial *ompA* gene sequencing, and that the strains were apparently associated to an individual farm. Indeed, sampling another flock of Breeder no. 5 confirmed the presence of one unique strain per farm based on partial *ompA* gene sequences (data not shown). In these circumstances, the source of infection remains unclear, but vertical transmission can be ruled out. It should be noted that the new *Chlamydophila* strains have also been detected in the "cou nu" breed of chicken (flocks 08-1274/3 and 08-1274/9). Interestingly, no *Chlamydiaceae* were found in guinea fowl ($n = 60$), although these birds were mixed with proven positive chickens (flocks 08-1274/2, 08-1274/3 and 08-1274/4).

Notably, no clinical signs were observed in animals of the respective flocks. Autopsy conducted on five birds in flocks 08-1274/21 and 08-1274/23, did not reveal any macroscopic lesions, despite the fact that PCR examination of spleen, liver, lung, and intestinal tissue samples demonstrated an intensive and dominant colonization of intestinal tissue with the new *Chlamydiaceae* spp. in comparison to the other organs that tested weakly positive (data not shown). This is in agreement with the observation of Gaede et al. (2008) that genetically related non-classified *Chlamydophila* spp. were found in symptomless chickens during an outbreak of clinical psittacosis.

The etiological importance of these new chlamydial isolates for human pneumonia has yet to be defined. So far, there has been no hard evidence of the strains being responsible for the three reported human cases of atypical pneumonia. Although clinical signs were reminiscent of psittacosis, i.e. high fever and pulmonary invasion, they cannot be regarded as specific. The three affected individuals were successfully treated with macrolide antibiotics (see Section 2). A possible approach to the assessment of the pathogenicity of the present strains could include experimental challenge trials in mice based on the protocol of Rodolakis et al. (1989).

Finally, it should be emphasized that detection of the presumed new members of *Chlamydiaceae* became possible because of the use of advanced yet complementary DNA-based diagnostic methods, i.e. real-time PCR in conjunction with the AT test. This combination, which was already suggested as a reference standard

(Sachse et al., 2009), can be further recommended for the laboratory diagnosis of animal and human chlamydial infections.

Conflict of interest statement

None of the authors (K. Laroucau, F. Vorimore, R. Aaziz, A. Berndt, E. Schubert, K. Sachse) has a financial or personal relationship with other people or organisations that could inappropriately influence or bias this paper.

Acknowledgments

We are grateful to Christine Grajetzki, Simone Bettermann, and Sabine Scharf who provided excellent technical assistance. We also thank Muriel Enique, Jean-Marie Gobin, Jean-Marie Arsicaud, Jérome Berthelot, David Leservoisier, Gilbert Marchand, Jacky Renaudin and the regional veterinary service for their precious help in flocks investigations and to Dr Loic Masson for his valuable help.

Part of the work conducted at Friedrich-Loeffler-Institut, Jena was funded by grant 01 KI 0720 "Zoonotic chlamydiae" from the Federal Ministry of Education and Research of Germany.

The study was an integral part of the European COST Action 855 "Animal chlamydioses and the zoonotic implications".

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.meegid.2009.08.005.

References

Borel, N., Kempf, E., Hotzel, H., Schubert, E., Torgerson, P., Slickers, P., Ehricht, R., Tasara, T., Pospischil, A., Sachse, K., 2008. Direct identification of chlamydiae from clinical samples using a DNA microarray assay—a validation study. Mol. Cell. Probes 22, 55–64.

Denamur, E., Sayada, C., Souriau, A., Orfila, J., Rodolakis, A., Elion, J., 1991. Restriction pattern of the major outer-membrane protein gene provides evidence for a homogeneous invasive group among ruminant isolates of *Chlamydia psittaci*. J. Gen. Microbiol. 137 (11), 2525–2530.

Ehricht, R., Slickers, P., Goellner, S., Hotzel, H., Sachse, K., 2006. Optimized DNA microarray assay allows detection and genotyping of single PCR-amplifiable target copies. Mol. Cell. Probes 20, 60–63.

Everett, K.D., Bush, R.M., Andersen, A.A., 1999. Emended description of the order *Chlamydiales*, proposal of *Parachlamydiaceae* fam. nov. and *Simkaniaceae* fam. nov., each containing one monotypic genus, revised taxonomy of the family *Chlamydiaceae*, including a new genus and five new species, and standards for the identification of organisms. Int. J. Syst. Bacteriol. 49 (Pt 2), 415–440.

Gaede, W., Reckling, K.F., Dresenkamp, B., Kenklies, S., Schubert, E., Noack, U., Irmscher, H.M., Ludwig, C., Hotzel, H., Sachse, K., 2008. *Chlamydophila psittaci* infections in humans during an outbreak of psittacosis from poultry in Germany. Zoonoses Public Health 55 (4), 184–188.

Harkinezhad, T., Geens, T., Vanrompay, D., 2009. *Chlamydophila psittaci* infections in birds: a review with emphasis on zoonotic consequences. Vet. Microbiol. 135 (1–2), 68–77.

Herrmann, B., Rubaiyat, R., Bergström, S., Bonnedahl, J., Olsen, B., 2000. *Chlamydophila abortus* in a brown skua (*Catharacta antarctica lonnbergi*) from a subantarctic island. Appl. Environ. Microbiol. 66, 3654–3656.

Kaleta, E.F., Taday, E.M., 2003. Avian host range of *Chlamydophila* spp. based on isolation, antigen detection and serology. Avian Pathol. 32 (5), 435–461.

Laroucau, K., Thierry, S., Vorimore, F., Blanco, K., Kaleta, E., Hoop, R., Magnino, S., Vanrompay, D., Sachse, K., Myers, G., Bavoil, P., Vergnaud, G., Pourcel, C., 2008a. High resolution typing of *Chlamydophila psittaci* by Multilocus VNTR Analysis (MLVA). Infect. Genet. Evol. 8 (2), 171–181.

Laroucau, K., Vorimore, F., Clerc, M., Capek, I., Garin-Bastuji, B., Bébéar, C., de Barbeyrac, B., 2008b. Les infections à *Chlamydophila psittaci* en France: données actuelles et enquête épidémiologique 2008–2009. Epidémiol. et Santé Anim. 54, 59–68.

Laroucau, K., de Barbeyrac, B., Vorimore, F., Clerc, M., Bertin, C., Harkinezhad, T., Verminnen, K., Obeniche, F., Capek, I., Bébéar, C., Vanrompay, D., Garin-Bastuji, B., Sachse, K., 2009. Chlamydial infections in duck farms associated with human cases of psittacosis in France. Vet. Microbiol. 135 (1–2), 82–89.

Pantchev, A., Sting, R., Bauerfeind, R., Tyczka, J., Sachse, K., 2008. New real-time PCR tests for species-specific detection of *Chlamydophila psittaci* and *Chlamydophila abortus* from tissue samples. Vet. J., doi:10.1016/j.tvjl.2008.02.025 2008 April 13 [Epub ahead of print].

Pudjatmoko, Fukushi, H., Ochiai, Y., Yamaguchi, T., Hirai, K., 1997. Phylogenetic analysis of the genus *Chlamydia* based on 16S rRNA gene sequences. Int. J. Syst. Bacteriol. 47 (2), 425–431.

Rodolakis, A., Bernard, F., Lantier, F., 1989. Mouse models for evaluation of virulence of *Chlamydia psittaci* isolated from ruminants. Res. Vet. Sci. 46 (1), 34–39.

Sachse, K., Grossmann, E., Jäger, C., Diller, R., Hotzel, H., 2003. Detection of *Chlamydia suis* from clinical specimens: comparison of PCR, antigen ELISA and culture. J. Microbiol. Method 54, 233–238.

Sachse, K., Hotzel, H., 2003. Detection and differentiation of chlamydiae by nested PCR. Methods Mol. Biol. 216, 123–136.

Sachse, K., Vretou, E., Livingstone, M., Borel, N., Pospischil, A., Longbottom, D., 2009. Recent developments in the laboratory diagnosis of chlamydial infections (Review). Vet. Microbiol. 135 (1–2), 2–21.

Spencer, W., Johnson, F., 1983. Simple transport medium for the isolation of *Chlamydia psittaci* from clinical material. Vet. Rec. 113, 535–536.

Thomas, V., Casson, N., Greub, G., 2006. *Criblamydia sequanensis*, a new intracellular *Chlamydiales* isolated from Seine river water using amoebal co-culture. Environ. Microbiol. 8 (12), 2125–2135.

Van Loock, M., Vanrompay, D., Herrmann, B., Vander Stappen, J., Volckaert, G., Goddeeris, B.M., Everett, K.D.E., 2003. Missing links in the divergence of *Chlamydophila abortus* from *Chlamydophila psittaci*. Int. J. Syst. Evol. Microbiol. 53, 761–770.

Vanrompay, D., Ducatelle, R., Haesebrouck, F., 1995. *Chlamydia psittaci* infections: a review with emphasis on avian chlamydiosis. Vet. Microbiol. 45, 93–119.

別紙様式第2-1

No. 27

医薬品 研究報告 調査報告書

識別番号・報告回数			報告日	第一報入手日	新医薬品等の区分	総合機構処理欄
				2009. 12. 20	該当なし	
一般的名称	新鮮凍結人血漿					
販売名(企業名)	新鮮凍結血漿「日赤」(日本赤十字社) 新鮮凍結血漿-LR「日赤」(日本赤十字社) 新鮮凍結血漿-LR「日赤」成分採血(日本赤十字社)		研究報告の公表状況	Satake M, Mitani T, Oikawa S, Nagumo H, Sugiura S, Tateyama H, Awakihara S, Mitsutomi Y, Muraoka M, Tadokoro K. Transfusion. 2009 Oct;49(10):2152-7.	公表国 日本	

研究報告の概要	○日本における初流血除去導入前後の血小板濃厚液の細菌汚染頻度 背景：血小板濃厚液(PC)の細菌汚染は、輸血医学における重大な感染リスクとなっている。細菌汚染を低減させる新しい戦略実施の必要性を評価するために、PCの正確な汚染頻度を明らかにすることが必要である。 試験デザインおよび方法：日本赤十字社が供給するPCの細菌汚染頻度を、初流血除去実施前後の有効期限切れPCを用いて調べた。偽陰性結果が可能な限り最少となる培養法をデザインした：保存から4日以上後に血小板検体をサンプリングし、好気性ボトルと嫌気性ボトルの双方に10mLの量を接種した。 結果：初流血除去実施前では、培養したPC21,786検体のうち36(0.17%)に細菌汚染があったことが確認された。実施後の汚染PC数は、21,783中11(0.05%)に減少し(減少率71%)、臨床的に重大な細菌の汚染の件数は、*Propionibacterium acnes*陽性PCを除き4件(0.018%)であった。献血者の血液に由来すると推定される細菌の汚染頻度は減少しなかった。 結論：初流血除去は細菌汚染頻度に確かな影響を及ぼす。西欧諸国と同等の細菌汚染頻度であり、培養スクリーニング検査が実施されていないにもかかわらず、日本でPC輸血後の敗血症反応発現率が低いことは、PCの保存期間が短い(72時間)ことが重要であると示唆される。
報告企業の意見	今後の対応
日本における献血血液の初流血除去導入についての評価報告である。血小板濃厚液の細菌汚染頻度は、初流血除去導入前後で0.17%から0.05%に減少し、臨床的に重大な細菌の汚染の件数は4件(0.018%)であった。培養スクリーニング検査が実施されていないにもかかわらず、輸血後の敗血症発現率が低いことは、PCの保存期間が短いことが重要であると示唆された日本赤十字社の安全対策に関する報告である。	日本赤十字社では、輸血による細菌感染予防対策として、すべての輸血用血液製剤を対象に保存前白血球除去及び初流血除去を導入している。さらに、輸血情報レーブレット等により細菌感染やウイルス感染について医療機関へ情報提供し注意を喚起しているほか、細菌感染が疑われる場合の対応を周知している。細菌やウイルスの検出や不活化する方策について検討している。

使用上の注意記載状況・その他参考事項等	研究報告の概要に関連する情報
新鮮凍結血漿「日赤」 新鮮凍結血漿-LR「日赤」 新鮮凍結血漿-LR「日赤」成分採血	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

Frequency of bacterial contamination of platelet concentrates before and after introduction of diversion method in Japan

Masahiro Satake, Takako Mitani, Shinji Oikawa, Hideto Nagumo, Sayoko Sugiura, Hidemi Tateyama, Syuji Awakihara, Yoshiro Mitsutomi, Masato Muraoka, and Kenji Tadokoro

BACKGROUND: Bacterial contamination of platelet concentrates (PCs) is the major infectious risk in transfusion medicine. To evaluate the necessity of implementing novel strategies for the reduction of bacterial contamination, it is necessary to establish a precise contamination frequency in PCs.

STUDY DESIGN AND METHODS: The frequency of bacterial contamination in PCs issued by the Japanese Red Cross was determined using expired PCs before and after the implementation of the diversion method. The culture method was designed such that it yields the least possibility of false-negative results: platelet specimens were sampled after at least 4 days of storage and the inoculum volume was 10 mL for both aerobic and anaerobic bottle cultures.

RESULTS: Of the 21,786 PCs cultured, 36 (0.17%) were confirmed to be bacterially contaminated before the implementation of the diversion method. After its implementation, the number of contaminated PCs decreased to 11 of 21,783 (0.05%) with a reduction rate of 71% and the number of contaminations of clinical importance was 4 (0.018%) excluding PCs positive for *Propionibacterium acnes*. The frequency of contamination by bacteria presumed to originate from donors' blood did not decrease.

CONCLUSION: The effect of the diversion method on the frequency of bacterial contamination is robust. The low incidence of septic reactions after PC transfusion in Japan in spite of the contamination frequency being comparable to those in Western countries and the non-institution of culture screening suggests the importance of a short shelf life (72 hr) for PCs introduced in Japan.

Bacterial contamination of blood components is the major residual infectious risk in modern transfusion medicine in developed countries. The transfusion of blood components with clinically relevant bacterial species at certain concentrations can lead to sepsis or a fatal outcome in transfusion recipients. This is particularly true for platelet concentrate (PC) products that are stored at 20 to 24°C. It is generally accepted that the frequency of bacterial contamination in PCs is approximately 1 in 3000.[1,2] To prevent transfusion-mediated septic reactions, several preventive measures have been proposed or implemented in each step from blood drawing to bedside practice of transfusion, namely, the improvement of the skin disinfection procedure,[3-5] use of a diversion pouch,[6-8] screening by culture for bacteria,[9-14] pH or glucose measurement, screening by amplification of a bacterial genome sequence,[15-17] and use of pathogen reduction/inactivation technologies.[18]

In Japan, all PC products are obtained using the apheresis system and the expiry time for PCs has been limited to 72 hours, which has undoubtedly contributed to the relatively infrequent occurrence of sepsis after PC transfusion. Over the past 8 years, however, two cases of septic reactions, including one fatal case, after PC transfusion have been confirmed by the Japanese Red Cross (JRC) blood center, and the necessity for the implementation of novel strategies for reducing bacterial contamination has been discussed. To this end, it is essential to establish a precise frequency of bacterial contamination in PCs processed under current regulations for blood procurement and processing. JRC systematically cultured more than 20,000 expired PCs using the culture conditions that were expected to provide the lowest possibility of false-negative results. JRC implemented the diversion method in October 2006 in the blood drawing process for PCs and reevaluated thereafter the bacterial contamination frequency in 20,000 expired PCs. In this article, we report the bacterial contamination frequency in PCs before and after the implementation of the diversion method and discuss the possible origin of bacteria detected in PCs obtained from otherwise healthy blood donors in Japan.

ABBREVIATIONS: JRC = Japanese Red Cross; PC(s) = platelet concentrate(s).

From the Japanese Red Cross Tokyo Metropolitan West Blood Center and the Japanese Red Cross Tokyo Metropolitan Blood Center, Tokyo; the Japanese Red Cross Hokkaido Blood Center, Hokkaido; the Japanese Red Cross Miyagi Blood Center, Miyagi; the Japanese Red Cross Aichi Blood Center, Aichi; the Japanese Red Cross Osaka Blood Center, Osaka; the Japanese Red Cross Okayama Blood Center, Okayama; the Japanese Red Cross Kyushu Blood Center, Fukuoka; and the Japanese Red Cross Blood Service Headquarters, Tokyo, Japan.

Address correspondence to: Masahiro Satake, Japanese Red Cross Tokyo Metropolitan West Blood Center, Midori-cho 3256, Tachikawa, Tokyo 190-0014, Japan; e-mail: ma-satake@tokyo.bc.jrc.or.jp.

Received for publication January 22, 2009; revision received March 18, 2009, and accepted March 31, 2009.

doi: 10.1111/j.1537-2995.2009.02243.x

TRANSFUSION 2009;49:2152-2157.

MATERIALS AND METHODS

Blood collection

In Japan, six types of PC categorized in accordance with the number of platelets (PLTs) contained have been approved, namely, Units 1, 2, 5, 10, 15, and 20. Unit 5 contains 1.0×10^{11} to 2.0×10^{11} PLTs and the number of PLTs contained in other units is in proportion to the unit number. Units 1 and 2 that are specifically used for infants or neonates, representing only 0.2% of all PCs, have been obtained from whole blood using the buffy coat method. All other units have been procured using the apheresis systems of CCS (Haemonetics, Inc., Tokyo, Japan), Terusys (Terumo, Inc., Tokyo, Japan), and Trima Accel (BCT Japan, Inc., Tokyo, Japan). In 2006, splitting of a larger unit was introduced for processing Units 1 and 2, and all PCs in Japan are now produced using the apheresis system. Unit 10 is the most frequently used, which contains 2.0×10^{11} to 3.0×10^{11} PLTs, representing approximately 80% of all PCs used in Japan.

For skin preparation for venipuncture, the donor's cubital fossa is cleansed by two courses of scrub with an isopropyl alcohol-containing cotton swab (One Shot plus, Hakujuji, Inc., Tokyo, Japan). Povidone-iodine alcohol (Isodine field solution, Meiji, Inc., Tokyo, Japan) is next applied on the area using a cotton-tipped applicator (Sterile Cotton Buds, Kawamoto, Inc., Osaka, Japan) in concentric circles away from the puncture site. After a minimum of 30 seconds of air drying, the donor's vein is punctured and blood is drawn. The diversion method was implemented in PC collection in October 2006 and in whole blood collection early in 2007. Twenty-five milliliters of initial flow of whole blood is collected in the integrated diversion pouch and the diverted blood is used for testing and blood archive registry.

Culture of expired PCs

There are 39 JRC blood centers all over Japan that process and distribute PCs. Among them, seven leading blood centers have laboratories with an automatic blood culture system, BacT/ALERT (Sysmex-bioMérieux, Tokyo, Japan). All PCs expiring in local blood centers after 72 hours of storage were further stored until Day 4 or 5 at 15 to 25°C and then sent to one of the leading laboratories. The PCs were maintained at 2 to 6°C during the transportation. The laboratories started PC culture between Day 4 and Day 9 but mostly did so on Day 5, 6, or 7. Common procedures were determined among the laboratories for sampling, inoculation, culturing, and retesting and were strictly followed by trained staff members; a PLT solution of more than 20 mL was drawn from the PC bag, of which 10 mL was inoculated into an anaerobic culture bottle and the remaining 10 mL into an aerobic bottle (BPN and BPA bottles, respectively, Sysmex-bioMérieux). All the procedures were conducted under aseptic conditions in a laminar air flow hood. The inoculated bottles were kept at 35°C in the BacT/ALERT system, and culture was continued until a positive signal was flagged or continued for 7 days in the absence of a positive signal. When a positive signal was flagged, culture was repeated using the original PC and frozen plasma obtained from the same donation. To confirm the bacterial species, bacteria-positive culture bottles were sent to the central laboratory of Tokyo Red Cross Blood Center and Tokyo Metropolitan Institute of Public Health.

Statistical analysis

The chi-square test was used to compare the bacterial contamination frequency of PCs procured before and after the implementation of the diversion method.

RESULTS

Frequency of bacterial contamination in PCs

During the period from May 2005 through April 2006 before the implementation of the diversion method, 21,786 expired PCs were cultured for bacterial examination. Culture started on Day 4, 5, 6, or 7 for 0.4, 20, 19, and 21% of PCs studied, respectively. There were 57 initial positive cultures, 10 of which were determined to have been caused by an inappropriate positive signal by the culture machine (Table 1). Of the remaining 47, 11 were defined as false-positive cultures on the basis of the negative result of the reculture of the aliquot from the original PC (Table 2). The number of confirmatory positive results

TABLE 1. Frequency of bacterial contamination in PCs collected with or without diversion method*

Variable	Without diversion	With diversion	Reduction (%) by diversion
Number of cultures	21,786	21,783	
Initially positive	57 (0.26)	23 (0.11)	
Machine failure	10	1	
False positive	11	11	
Confirmatory positive	36† (0.17)	11 (0.050)	71 (p = 0.0003)
Anaerobic only‡ (*P. acnes*)	24 (0.11)	7 (0.032)	71 (p = 0.004)
Aerobic and anaerobic§	13 (0.060)	4 (0.018)	70 (p = 0.052)

* Data are reported as number (%) unless otherwise specified.
† One culture was doubly contaminated by *P. acnes* and *Staphylococcus* sp.
‡ All the cultures that were anaerobic bottle positive and aerobic bottle negative were identified to be contaminated by *P. acnes*.
§ All the cultures positive for bacteria using aerobic culture bottle were identified to be also positive using anaerobic culture bottle.

TABLE 2. Bacterial species determined as false positive by repeat negative culture

Without diversion (n = 11)	With diversion (n = 11)
P. acnes (7)*	*P. acnes* (8)†
Bacillus sp. (2)	*Bacillus* sp. (2)
Brevibacillus choshinensis (1)	*Bacillus circulans* (1)
Staphylococcus saccharolyticus (1)	

* The number of cultures initially positive for *P. acnes* was 31.
† The number of cultures initially positive for *P. acnes* was 15.

was 36, representing 0.17% of all PCs cultured. Of these 36, 24 (0.11%) were identified only by anaerobic bottle culture and the bacterial species were all determined to be *Propionibacterium acnes* (Table 1). Thirteen (0.060%) were identified both by aerobic and anaerobic bottle culture. One PC was cocontaminated by *Staphylococcus* sp. and *P. acnes*. Eight non-*P. acnes* bacterial species were considered to be derived from the donors' skin and three from the donors' peripheral blood (Table 3). The remaining two PCs were contaminated by *Staphylococcus aureus* and could be derived either from transient skin flora or the donors' blood stream. *P. acnes* was initially detected in 31 PCs but was not detected in 7 PCs by repeated culture (Tables 2 and 3).

Two months after the introduction of the diversion method, when the staff in every donation site became proficient with the procedure, the culture study of expired PCs restarted in December 2006 and ended with the culture of 21,783 PCs in March 2008. Culture started on Day 4, 5, 6, or 7 for 0, 19, 21, and 24% of PCs studied, respectively. There was no significant difference in the date of start of culture between before and after diversion method. Table 1 shows the results of the study. Twenty-three were initially positive, of which one was caused by machine failure as described previously, 11 were false positive, and 11 were determined as confirmatory positive, representing 0.050% of all cultured PCs. Seven (0.032%) of the 11 PCs were found to be contaminated by *P. acnes* only by anaerobic bottle culture. The remaining four PCs were found to be contaminated by other species both by aerobic and by anaerobic bottle culture, indicating that PCs currently released from JRC blood centers after implementation of the diversion method have a 0.018% frequency of contamination by bacteria other than *P. acnes*. One of the four PCs was positive for *Staphylococcus epidermidis* that could be derived from the donors' skin, two were positive for *Streptococcus dysgalactiae* subsp. *equisimilis* and *Escherichia coli* that could be derived from the donors' peripheral blood, and one was positive for *S. aureus* that could be derived from either origin (Table 3). Our culture study of more than 40,000 expired PCs confirmed that the diversion of initial blood flow into the integrated pouch decreased the contamination rate for all bacterial species by 71% (p = 0.0003 by chi-square test, Table 1).

DISCUSSION

The frequency of bacterial contamination in PCs has been recently studied to evaluate the residual risks in the context of the implementation of culture screening. Most of them report the results obtained from routine culture screening conducted as a release test. There are, however, some limitations in those studies regarding the sensitivity of the culture method used, that is, the limited incubation time before sampling and the limited sample volume inoculated into culture bottles. In this study, the culture procedures employed were designed such that the possibility of false-negative results could be as low as possible: the storage period of PCs at 15 to 25°C before sampling was 4 days at the minimum; PCs were stored at a low temperature after 5 days of storage to prevent autolysis of fully grown bacteria; culture was conducted using anaerobic as well as aerobic bottles; the inoculation volume was 10 mL, which is the maximum volume for each culture bottle; and culture was continued for 7 days. Accordingly, the frequencies of bacterial contamination described in this article would be the highest values obtainable using available techniques for bacterial detection with minimal possibility of false-negative results, although possibility remains that false-negative results occur if bacteria die in PCs with storage.

TABLE 3. Confirmed bacterial species detected in PCs other than *P. acnes*

Estimated origin of bacteria	Without diversion	With diversion
Skin flora	*S. epidermidis* (4)*	*S. epidermidis* (1)
	Staphylococcus sp. (1)	
	CNS† (1)	
	S. saccharolyticus (1)	
	Gram(+) bacillus, nonspore (1)	
Transient skin flora or blood	*S. aureus* (2)	*S. aureus* (1)
Peripheral blood	*S. constellatus* (1)	*Streptococcus dysgalactiae* subsp. *equisimilis* (1)
	Salmonella serovar Choleraesuis (1)	*E. coli* (1)
	E. corrodens (1)	

* Number in parentheses is number of cases.
† CNS = coagulase-negative *Staphylococcus*.

Overall, the implementation of the diversion method significantly reduced the frequency of bacterial contamination in PCs by 71% for all bacterial species (Table 1). If the results obtainable using only aerobic culture bottles are considered, the contamination rates without or with the diversion method was 0.06 and 0.018%, respectively, both of which are the values comparable to those reported in Western countries.[1,9,10,13]

In Japan, approximately 700,000 PCs are released and transfused to patients every year. From the calculation based on the data shown in Table 1, it is estimated that as many as 770 PCs contaminated by *P. acnes* had been released every year from blood centers before the diversion method was implemented. Through the JRC hemovigilance, however, there has been no report of adverse reactions after PC transfusion that implicated the involvement of *P. acnes* contamination. In fact, *P. acnes* has been rarely reported to be of clinical significance in the literature.[18] Possible reasons for these are 1) the clinical virulence of *P. acnes* is usually considered to be very low, 2) PC bags currently used serve the suboptimal culture conditions for *P. acnes* in terms of oxygen delivery, and 3) the growing speed of *P. acnes* in PC bags is very low.[20] In this study, the time required for obtaining the positive signal using BacT/ALERT was 3.14 to 6.83 days (mean, 4.7 days) from the start of culture, indicating that it took a minimum of 7 days from PC donation, far exceeding the shelf life of PCs in Japan. The frequency of contamination with clinical relevance could, therefore, be expressed excluding *P. acnes*-contaminated PCs, namely, 0.018% or 126 products per year after the use of the diversion pouch. The significance of using anaerobic culture bottles here would be that the sample volume is doubled and that it often shows better sensitivity than using aerobic culture bottles.[21]

Jacobs and colleagues[22] calculated the rate of septic reactions after the transfusion of bacterially contaminated components as 41% and the rate of fatality among the septic reactions as 11% on the basis of their elaborate prospective study. With these figures and the contamination rate obtained from this study, the total number of septic reactions and the fatality in Japan are estimated as 52 and 5.7 per year, respectively. However, most of the PCs that Jacobs and colleagues described as contaminated are considered to have had a high bacterial load because the sensitivity of the culture method they used was relatively low (10 colony-forming units/mL) and some PCs that were contaminated were identified while they evaluated only PCs stored for 4 days or more. Moreover, most of the contaminated PCs were transfused 4 and 5 days after donation. Therefore, the rates of septic reactions and fatality described in their study are considered to be the results of the transfusion of PCs, most of which were heavily contaminated. The contaminated PCs in our study could include those that had a low bacterial load if they were transfused within the 3-day shelf life. The estimated frequencies of septic reactions and fatality in Japan that were described previously could, in this context, be overestimated.

After the implementation of the diversion method, the number of contaminated PCs possibly originating from the donors' skin flora except for *P. acnes* decreased markedly from eight to one (p = 0.046, Table 3). On the other hand, the contamination possibly caused by the bacteria from the donors' peripheral blood or transient skin flora remains to be a serious problem, showing a decrease in the number of contaminated PCs from five to three. These observations substantiate the theoretical mechanism of the effect of the method of initial flow diversion.

Through the extensive culture of more than 40,000 expired PCs, we identified several bacterial species that could have caused a serious clinical outcome if PCs contaminated at clinically relevant concentrations were transfused. Both *Streptococcus constellatus* and *Eikenella corrodens* sometimes cause periodontitis, local abscess, sepsis, or meningitis and are frequently found in the oral cavity or upper respiratory tract. JRC experienced a fatal case of sepsis caused by the transfusion of a PC contaminated by *Streptococcus pneumoniae*,[23] which must have been derived from the donor's upper respiratory tract or oral cavity. These observations suggest that the transient bacteremia caused by bacterial invasion into the blood

stream from the oral cavity, periodontal space, or upper respiratory tract is not a rare event but that people with such bacteremia represent a considerable proportion of otherwise healthy blood donors.

Three PCs contaminated by *S. aureus* were identified during the culture study. Through donor interview, it was verified that two of the three had atopic dermatitis on their cubital fossa or face. *S. aureus* has been implicated in bacteremia or sepsis in patients with atopic dermatitis[24,25] and it is possible that the organism invaded the donors' peripheral blood from their skin lesion and eventually contaminated PCs. Skin lesions such as atopic dermatitis may serve as a risk factor for bacteremia in blood donors regardless of whether the lesion is on the venipuncture site or not.

In spite of the considerably high rate of bacterial contamination of PCs, only two septic cases including one fatality have been confirmed over the past 8 years from 2000 through 2007. These figures are smaller than those reported by Eder and coworkers[4] for rate of fatality (1/500,000) and septic reactions (1/75,000) after the implementation of universal culture screening of PCs. The most likely reason for it is that the shelf life of PCs has been limited to 72 hours in Japan. We believe that administration of PCs as soon as possible after processing will remain as the best strategy of preventing transfusion-related sepsis whatever new strategies for the reduction of bacterial contamination in PCs are implemented in the near future, although we detected contamination with *E. coli* and *S. aureus*, two organisms that can grow rapidly in PLT products and lead to life-threatening reactions within 3 days of collection. Another reason may be that all PC products in Japan are obtained using the apheresis system, which would decrease the contamination rate in theory, although some recent articles argue for the similarity of contamination rate for apheresis PLTs and pooled PLTs.[3,26] It is also possible that low sensitivity of the current hemovigilance system contributes to the low rate of septic reactions in Japan. It is, however, highly likely that at least the fatality rate obtained represents a real occurrence, as suggested by Jacobs and coworkers,[22] because it is mandatory in Japan for clinicians to report any serious transfusion-related adverse effect to the Ministry of Health, Labour and Welfare, and every such event is also evaluated by the JRC headquarters.

The introduction of culture screening is unlikely in JRC blood centers because it will oblige us to extend the shelf life to 5 days, which would trade off the real merit of the currently short shelf life of PCs. To determine whether the implementation of a novel strategy such as pathogen inactivation/reduction[27,28] or point-of-issue testing of PCs using a rapid assay[29] is indispensable, it is essential to establish the data for patients to be rescued with the new strategy and the cost required for the institution and maintenance of the new strategy.

CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest relevant to the manuscript submitted to TRANSFUSION.

REFERENCES

1. Blajchman MA, Beckers EA, Dickmeiss E, Lin L, Moore G, Muylle L. Bacterial detection of platelets: current problems and possible resolutions. Transfus Med Rev 2005;19: 259-72.
2. Hillyer CD, Josephson CD, Blajchman MA, Vostal JG, Epstein JS, Goodman JL. Bacterial contamination of blood components: risks, strategies, and regulation: joint ASH and AABB educational session in transfusion medicine. Hematology (Am Soc Hematol Educ Program) 2003; 575-89.
3. de Korte D, Curvers J, de Kort WL, Hoekstra T, van der Pool CL, Beckers EA, Marcelis JH. Effect of skin disinfection method, deviation bag, and bacterial screening on clinical safety of platelet transfusions in the Netherlands. Transfusion 2006;46:476-85.
4. McDonald CP, Lowe P, Roy A, Robbins S, Hartley S, Harrison JF, Slapecki A, Verlander N, Barbara JA. Evaluation of donor arm disinfection techniques. Vox Sang 2001; 80:135-41.
5. Goldman M, Roy G, Fréchette N, Décary F, Massicotte L, Delage G. Evaluation of donor skin disinfection methods. Transfusion 1997;37:309-12.
6. Kojima K, Togashi T, Hasegawa K, Kawasaki H. Subcutaneous fatty tissue can stray into a blood bag. Vox Sang 1998;74 Suppl 1:1205.
7. Wagner SJ, Robinette D, Friedman L, Miripol J. Diversion of initial blood flow to prevent whole-blood contamination by skin surface bacteria: an in vitro model. Transfusion 2000;40:335-8.
8. Bruneau C, Perez P, Chassaigne M, Allouch P, Audurier A, Gulian C, Janus G, Boulard G, De Micco P, Salmi LR, Noel L. Efficacy of a new collection procedure for preventing bacterial contamination of whole-blood donations. Transfusion 2001;41:74-81.
9. Eder AF, Kennedy JM, Dy BA, Notari EP, Weiss JW, Fang CT, Wagner S, Dodd RY, Benjamin RJ, American Red Cross Regional Blood Centers. Bacterial screening of apheresis platelets and the residual risk of septic transfusion reactions: the American Red Cross experience (2004-2006). Transfusion 2007;47:1134-42.
10. Kleinman SH, Kamel HT, Harpool DR, Vanderpool SK, Custer B, Wiltbank TB, Nguyen KA, Tomasulo PA. Two-year experience with aerobic culturing of apheresis and whole blood-derived platelets. Transfusion 2006;46:1787-94.
11. Ramirez-Arcos S, Jenkins C, Dion J, Bernier F, Delage G, Goldman M. Canadian experience with detection of bacterial contamination in apheresis platelets. Transfusion 2007; 47:421-9.
12. Munksgaard L, Albjerg L, Lillevang ST, Gahrn-Hansen B, Georgsen J. Detection of bacterial contamination of platelet components: six years' experience with the BacT/ALERT system. Transfusion 2004;44:1166-73.
13. Larsen CP, Ezligini F, Hermansen NO, Kjeldsen-Kragh J. Six years' experience of using the BacT/ALERT system to screen all platelet concentrates, and additional testing of outdated platelet concentrates to estimate the frequency of false-negative results. Vox Sang 2005;88:93-7.
14. Fang CT, Chambers LA, Kennedy J, Strupp A, Fucci MC, Janas JA, Tang Y, Hapip CA, Lawrence TB, Dodd RY, American Red Cross Regional Blood Centers. Detection of bacterial contamination in apheresis platelet products: American Red Cross experience, 2004. Transfusion 2005;45: 1845-52.
15. Kunishima S, Inoue C, Nishimoto Z, Kamiya T, Ozawa K. Application of 16s ribosomal RNA gene amplification to the rapid identification of bacteria from blood culture bottles. Transfusion 2000;40:1420-1.
16. Reesink HW, Mohammadi T, Pietersz RN, Savelkoul PH. Rapid screening by real-time 16S rDNA PCR for bacterial contamination of blood products. Clin Chem Lab Med 2008;46:954-62.
17. Dreier J, Störmer M, Kleesiek K. Real-time polymerase chain reaction in transfusion medicine: applications for detection of bacterial contamination in blood products. Transfus Med Rev 2007;21:237-54.
18. Klein HG, Anderson D, Bernardi MJ, Cable R, Carey W, Hoch JS, Robitaille N, Sivilotti ML, Smaill F. Pathogen inactivation: making decisions about new technologies. Report of a consensus conference. Transfusion 2007;47: 2338-47.
19. Wendel S, Fontao-Wendel R, Germano S, Morato LE, Lazar A, Sakashita A. Screening of bacterial contamination in a routine scale for blood components production in a Brazilian blood bank. Vox Sang 2000;78 Suppl 1:P376.
20. Störmer M, Kleesiek K, Dreier J. *Propionibacterium acnes* lacks the capability to proliferate in platelet concentrates. Vox Sang 2006;91:193-201.
21. Brecher ME, Hay SN. Investigation of an isolate of *Staphylococcus lugdunensis* implicated in a platelet fatality: a possible advantage of the use of an anaerobic bottle. Transfusion 2007;47:1390-4.
22. Jacobs MR, Good CE, Lazarus HM, Yomtovian RA. Relationship between bacterial load, species virulence, and transfusion reaction with transfusion of bacterially contaminated platelets. Clin Infect Dis 2008;46:1214-20.
23. Katayama T, Kamiya M, Hoshina S, Masuoka H, Nishiwaki K, Sano K, Hagino T, Kobayashi M. Fatal septic shock and rhabdomyolysis following transfusion of platelet concentrates contaminated with *Streptococcus pneumoniae*. Rinsho Ketsueki 2003;44:381-5.
24. Hoeger PH, Ganschow R, Finger G. Staphylococcal septicemia in children with atopic dermatitis. Pediatr Dermatol 2000;17:111-4.
25. Tomi NS, Kränke B, Aberer E. Staphylococcal toxins in patients with psoriasis, atopic dermatitis, and erythroderma, and in healthy control subjects. J Am Acad Dermatol 2005;53:67-72.
26. Schrezenmeier H, Walther-Wenke G, Müller TH, Weinauer F, Younis A, Holland-Letz T, Geis G, Asmus J, Bauerfeind U, Burkhart J, Deitenbeck R, Förstemann E, Gebauer W, Höchsmann B, Karakassopoulos A, Liebscher UM, Sänger W, Schmidt M, Schunter F, Sireis W, Seifried E. Bacterial contamination of platelet concentrates: results of a prospective multicenter study comparing pooled whole blood-derived platelets and apheresis platelets. Transfusion 2007;47:644-52.
27. Cazenave JP. European view about pathogen inactivation in labile blood products. Vox Sang 2008;95 Suppl 1:8.
28. Osselaer JC, Messe N, Hervig T, Bueno J, Castro E, Espinosa A, Accorsi P, Junge K, Jacquet M, Flament J, Corash L. A prospective observational cohort safety study of 5106 platelet transfusions with components prepared with photochemical pathogen inactivation treatment. Transfusion 2008;48:1061-71.
29. Woodbury C, Stiles M, Lajoie C, Best N, Sisson J, Proctor K, Hall JA. Performance of platelet PGD® test using leuko-reduced apheresis units. Transfusion 2007;47 Suppl: SP485. ◘