別紙様式第2

別紙(2)-3

医薬品　研究報告　調査報告書

識別番号・報告回数		報告日	第一報入手日 2009年1月21日	新医薬品等の区分 該当なし	厚生労働省処理欄
一般的名称	①～③、⑥～⑧人血清アルブミン ④⑨人血液凝固第 XIII 因子 ⑤フィブリノゲン加第 XIII 因子	研究報告の公表状況	*Babesia* Infection through Blood Transfusions: Reports Received by the US Food and Drug Administration, 1997–2007 Clinical Infectious Diseases, 1 January 2009, Vol. 48, No. 1: pp. 25-30	公表国 米国	
販売名（企業名）	①アルブミンーベーリング②アルブミナー5% ③アルブミナー25%④フィブロガミン P⑤ベリブラスト P コンビセット⑥アルブミン-ベーリング 20%静注 10.0g/50mL⑦アルブミナー5%静注 12.5g/250mL⑧アルブミナー25%静注 12.5g/50mL⑨フィブロガミン P 静注用（CSL ベーリング株式会社）				

研究報告の概要

問題点（輸血によるバベシア感染による死亡報告）

FDA は血液収集や輸血の合併症疑いに関する情報を、供血者及び受血者の死亡報告、副作用報告システム（MedWatch を含む）、生物学的製剤逸脱報告システム（Biological Product Deviations Reporting System: BPDR）の安全性調査システムにより入手している。FDA は 1996 年 10 月 1 日から 2007 年 12 月 31 日に報告されたバベシア症の傾向を評価するため、これらのシステムを照会し、分析した。

FDA は 2005 年に 2 例、2006 年に 3 例、2007 年に 3 例の供血者及び受血者のバベシア症による死亡報告を受けていた。臨床経過は、無脾症患者、免疫不全患者や他の内科の慢性疾患患者に発症したダニ媒体バベシア感染症と一致していた。全員が B.microti に感染し、赤血球輸血を受けていた。受血者は輸血後 2.5-7 週で症状が進展し、輸血後 2 ヶ月以内に死亡した。

FDA は各死亡例が医学的な検討で輸血によるバベシア症であるとしている。

BPDR において、輸血関連のバベシア感染疑い例と供血後のバベシア症感染は 1999 年の 0 件から 2007 年の 25 件に増加していた。副作用報告システムでは 1997 年から輸血によるバベシア感染は報告されていなかった。

各死亡例を蛍光抗体法で測定すると B.microti 抗体価は 128 倍以上であった。これらデータにより輸血によるバベシア感染は増加していることが示唆された。バベシア種は血液銀行の製造工程である冷凍、白血球除去やろ過の工程で生存できるため、赤血球、脱グリセル赤血球や血小板の輸血により病原体が感染する。症状発現から死亡まで短期間（5-17 日）であることを考慮して、輸血後の最初の数週間に起きる原因不明の発熱を評価するため、特に無脾症患者や免疫不全患者では、末梢血塗抹標本の試験などでバベシア種を早期に検討すべきである。バベシア症はアメリカで届出義務はないが、輸血によるバベシア感染を当局に報告することにより感染供血者を特定し、残存している血液の使用を禁止することができる。

血液収集者は、潜在的に感染している使用期限内の血液成分を速やかに廃棄するため、直ちに供血後のバベシア症について輸血を実施する施設に報告すべきである。また血液事業者は、死亡報告及び BPDR を FDA に報告すべきである。

以上のことから、輸血によるバベシア感染は増加していることが示された。

報告企業の意見	今後の対応
バベシア症は赤血球等内にバベシア原虫が寄生するため発症するが、本剤は血漿を原材料にしているため感染はないと考えられる。	今後とも新しい感染症に関する情報収集に努める所存である。

使用上の注意記載状況・その他参考事項等

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Babesia Infection through Blood Transfusions: Reports Received by the US Food and Drug Administration, 1997–2007

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Background. Human babesiosis is an illness with clinical manifestations that range from asymptomatic to fatal. Although babesiosis is not nationally notifiable, the US incidence appears to be increasing. *Babesia* infection is a transfusion-transmissable disease. An estimated 70 cases were reported during 1979–2007; most of these cases were reported during the past decade.

Methods. We queried the 3 following US Food and Drug Administration safety surveillance systems to assess trends in babesiosis reporting since 1997: fatality reports for blood donors and transfusion recipients, the Adverse Event Reporting System (which includes MedWatch), and the Biological Product Deviations Reporting system. We analyzed fatality reports for time frames, clinical presentations, and patient and donor demographic characteristics.

Results. Eight of 9 deaths due to transfusion-transmitted babesiosis that were reported since 1997 occurred within the past 3 years (2005–2007). Four implicated donors and 5 patients lived in areas where *Babesia* infection is not endemic. Increasing numbers of Biological Product Deviations Reports were submitted to the US Food and Drug Administration over the past decade; the Adverse Event Reporting System received no reports.

Conclusions. After nearly a decade with no reported death due to transfusion-transmitted babesiosis, the US Food and Drug Administration received 8 reports from November 2005 onward. The increased numbers of deaths reported and Biological Product Deviations Reports suggest an increasing incidence of transfusion-transmitted babesiosis. Physicians should consider babesiosis in the differential diagnosis in immunocompromised, febrile patients with a history of recent transfusion, even in areas where *Babesia* infection is not endemic. Accurate and timely reporting of babesiosis-related donor and transfusion events assists the US Food and Drug Administration in developing appropriate public health–control measures.

Human babesiosis is a protozoal zoonotic illness that is transmitted primarily by *Ixodes scapularis* ticks in North America. Of >100 *Babesia* species that infect vertebrate hosts, *Babesia microti*, *Babesia divergens*–like organisms, *Babesia duncani* (previously known as WA-1), CA-1, and MO-1 infect humans in the United States [1]. The majority of US babesiosis cases are attributed to *B. microti*, which is found mostly in the northeastern and upper midwestern states.

Received 19 August 2008; accepted 1 October 2008; electronically published 26 November 2008.

Data in this article are based on information provided to the US Food and Drug Administration in required reports of potentially transfusion-related deaths.

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Clinical Infectious Diseases 2009; 48:25–30

1058-4838/2009/4801-0004

DOI: 10.1086/595010

Clinical manifestations range from mild, self-limited flu-like symptoms to severe malaise, fatigue, fever, anorexia, arthralgia, myalgia, depression, vomiting, and anemia. Complications can include acute respiratory failure, congestive heart failure, and renal failure [2, 3]. Patients who are immunocompromised, asplenic, coinfected with other tick-transmitted infectious pathogens, and/or elderly are at risk of increased disease severity [1, 4, 5].

After acquiring *Babesia* parasites from a tick bite, infected individuals may develop symptoms within 1–4 weeks. Most cases are probably not reported, because many infections are asymptomatic; symptoms are mild, or a patient may be coinfected with *Borrelia burgdorferi* (with *Babesia* infection remaining undiagnosed) [6–8]. In addition to a probable lack of clinical awareness, especially in areas of nonendemicity, many states have

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no reporting requirement [6, 9, 10], and babesiosis, unlike Lyme disease, is not nationally notifiable. Infected patients can harbor circulating parasites for months or years without symptoms; patients with chronic low-level parasitemia may unknowingly transmit the organisms through donating blood [7, 8]. There is no licensed test for *Babesia* screening of donated blood products.

The majority of an estimated 70 transfusion-transmitted *Babesia* infections since 1979 involved *B. microti*; most of these infections were reported in the past decade (D. Leiby, personal communication) [7]. The national standard blood donor questionnaire includes questions about prior babesiosis infection and general donor health [11]. Individuals with previously diagnosed babesiosis are indefinitely deferred (ineligible to donate blood). However, mild *Babesia* infections may remain unrecognized, and infected individuals may not recall recent tick bites [12].

The purpose of this article is to alert clinicians and the public health community of reported deaths related to transfusion-transmitted babesiosis; to describe the US Food and Drug Administration's (FDA's) surveillance systems for adverse events and product manufacturing deviations related to donor blood collection, distribution, and transfusion; and to encourage the reporting of suspected cases of transfusion-transmitted babesiosis.

METHODS

The FDA's surveillance systems. The FDA receives information about suspected complications of blood collection and transfusion via the 3 following systems: fatality reports for blood donors and transfusion recipients, the Adverse Event Reporting System (AERS; which includes the FDA MedWatch program), and the Biological Product Deviations Reporting (BPDR) system (table 1).

Blood establishments are required to notify the FDA "when a complication of blood collection or transfusion is confirmed to be fatal" [13, p. 58]. Center for Biologics Evaluation and Research medical officers review documentation from the reporting facility and reports from FDA investigators to assess the relationship, if any, to the blood donation or transfusion.

Biologics manufacturers are required to submit reports of adverse experiences to the AERS, the FDA's computerized database for postmarketing safety surveillance. The voluntary MedWatch program allows health care professionals and consumers to report adverse events to the AERS.

The FDA's BPDR system receives reports of "any event...associated with the manufacturing, to include testing, processing, packing, labeling, or storage, or with the holding or distribution of a licensed biological product or blood or blood components... in which the safety, purity, or potency of a distributed product may be affected" [14].

Data query. We queried these systems for babesiosis-related blood donation or transfusion events reported from 1 October 1996 (FDA fiscal year 1997) through 31 December 2007 (first quarter of fiscal year 2008). We analyzed fatality reports for time frames, clinical presentations, and patient and donor demographic characteristics. Babesiosis-related reports to the BPDR system typically describe either possible transfusion-transmitted disease or postdonation illness, with potential implications for the safety of the donated blood units. We categorized cases reported to the BPDR system as postdonation illness and potential transfusion transmission–related events. To avoid distortion of BPDR trends, we excluded reports of infected donors identified prospectively through antibody assay research [7].

RESULTS

Reported deaths of blood donors and recipients. Before 2005, the FDA received the last fatality report of transfusion-transmitted babesiosis in 1998; there were 2 reports in 2005, 3 in 2006, and 3 in 2007. Clinical presentations (table 2) were consistent with natural tick-borne *Babesia* infection in asplenic, immunocompromised, or other patients with serious comorbid chronic disease [12]. All were infected with *B. microti* and had received RBCs; 1 death was attributable to a unit of frozen deglycerolized RBCs. Recipients developed symptoms in 2.5–7 weeks and died within 2 months after transfusion of the implicated blood units (table 3). FDA medical review verified that transfusion-transmitted babesiosis contributed to each death.

BPDR. Figure 1 summarizes 10 years of BPDRs for potential transfusion-transmitted *Babesia* infection and postdonation babesiosis. The numbers that were received range from 0 in fiscal year 1999 to 25 in fiscal year 2007.

AERS. Since 1997, the AERS has not received any report of transfusion-transmitted babesiosis.

Laboratory and blood establishment investigations. All fatal cases (in blood recipients) reported here were initially diagnosed with use of a thin peripheral blood smear. For each fatality, subsequent donor testing by immunofluorescence antibody assay revealed elevated *B. microti* antibody titers (≥1:128). All implicated donors were indefinitely deferred from donating blood.

DISCUSSION

Babesiosis has gained attention as an emerging zoonotic disease with an expanding known geographical range [6, 9, 15, 16]. Since November 2005, the FDA learned of 8 deaths involving transfusion-transmitted babesiosis and has received increasing reports of nonfatal cases and postdonation illness. Because of the likelihood of underreporting to the FDA's surveillance systems, these data suggest that the incidence of transfusion-transmitted babesiosis may be increasing.

Table 1. US Food and Drug Administration (FDA) surveillance systems for biologics.

Surveillance system	Regulatory authority	Products covered	Reporting entity	Additional information	Publicly accessible data
Fatalities	Required per 21 CFR 606.170(b)	Blood and blood-product collection and transfusion	Blood establishments	Guidance: "Notifying the FDA of Deaths Related to Blood Collection or Transfusion" (http://www.fda.gov/cber/gdlns/bldfatal.htm)	Annual summaries (http://www.fda.gov/cber/blood/fatal0506.htm)
AERS	Required per 21 CFR 600.80	Drugs and therapeutic biologics	Manufacturer	http://www.fda.gov/cder/aers/default.htm	Quarterly data files (http://www.fda.gov/cder/aers/extract.htm)
MedWatch	Voluntary	Blood, blood products, biologics, and drugs	Health care professionals and consumers	http://www.fda.gov/medwatch/report/hcp.htm	Quarterly data in AERS files (http://www.fda.gov/cder/aers/extract.htm)
BPDR	Required per 21 CFR 600.14 and 21 CFR 606.171	Blood and blood products	Blood establishments	http://www.fda.gov/cber/fhiodev/biodev.htm	Annual summaries (http://www.fda.gov/cber/biodev/reports.htm)

NOTE. AERS, Adverse Reporting System; BPDR, Biological Product Deviations Reports; CFR, Code of Federal Regulations.

Table 2. Summary of deaths attributed to transfusion-transmitted *Babesia* infection that were reported to the US Food and Drug Administration.

Patient	Age, years	Sex	State of residence	Medical history	Presenting complaint	Clinical course	Donor information
1	81	Female	Maryland	Hypercholesterolemia, hypertension, severe nosebleeds requiring transfusion but otherwise in good health	Severe fatigue and lethargy	CBC showing a hemocrit of 21%, a platelet level of 21,000 platelets/mL, BUN level of 80 mg/dL, and a creatinine level of 2.5 mg/dL, indicating renal failure; examination for anemia and fatigue identified *Babesia* species on PB smear (positive PCR result); treated with quinine and clindamycin; developed signs of adult respiratory distress syndrome; experienced thrombotic cerebrovascular accident on day 5 of treatment with high fever	Resident of Maryland; traveled to New York (Long Island); positive PB smear result; *B. microti* IFA titer of 1:512
2	88	Male	Rhode Island	Chronic myelomonocytic leukemia with chronic anemia (transfusion dependent) and GI bleed	4-Day history of progressive weakness, fatigue, and anorexia with low-grade fever	*Babesia* species identified by PB smear; treated with atovaquone and azithromycin; died on hospital day 12 with persistent parasitemia, progressive renal failure, anemia, and altered mental status	Resident of Rhode Island; *B. microti* IFA titer of 1:1024
3	57	Male	Texas	Recent history of melena and previous hepatitis B and C virus infection, cirrhosis, coronary artery disease, congestive heart failure, receipt of coronary artery bypass grafts, and GI bleed requiring transfusion	10–12-Day history of fever, night sweats, chills, and other complaints of melena, weakness, dizziness, anorexia, and increasing ascites	*Babesia* species identified by PB smear; treated; developed altered mental status, respiratory distress and GI bleed	Resident of Texas; history of travel to Massachusetts; *B. microti* IFA titer of 1:256
4	76	Male	Minnesota	Transfusion-dependent acute myelocytic leukemia, rheumatoid arthritis, steroid-induced immunosuppression, and history of splenectomy, coronary artery disease, idiopathic thrombocytopenia, and multiple other medical problems	Several-day history of fever, cough, weakness, and dyspnea	Sepsis examination and broad-spectrum antibiotics started at hospital admission, with *Babesia* infection diagnosed (by PB smear) and treated on hospital day 2; death due to multiple medical problems	Resident of Minnesota; *B. microti* IFA titer of 1:256; negative PCR result
5	71	Female	Connecticut	Chronic liver disease (portal hypertension with gastroesophageal varices and hepatorenal syndrome), chronic transfusion-dependent anemia and diabetes, splenectomy, and cholecystectomy	Low-grade fever, complaints of chills and weakness for several days, with hemocrit decreasing from 29% to 23% at routine outpatient CBC monitoring	*Babesia* species identified by PB smear; treated; developed acute tubular necrosis, altered mental status, and progressive hypotension	Resident of Connecticut; *B. microti* IFA titer of 1:256; positive Western Blot result; negative PCR result
6	82	Female	Ohio	Receipt of coronary artery bypass grafts and aortic valve replacement with transfusion, atrial fibrillation, cerebrovascular accident, and hyperlipidemia	Several-day history of fever and chills, with anemia and thrombocytopenia diagnosed at hospital admission	*Babesia* species identified by PB smear; treated with clindamycin and quinine *plus* automated RBC exchange by apheresis, which reduced parasitemia from 26% to 5%; developed altered mental status; the patient died of multiple-organ failure, *Staphylococcus aureus* pneumonia, and acute myocardial infarction	Resident of Ohio; traveled to Connecticut 2 months before donating blood; *B. microti* IFA titer of 1:256
7	74	Female	New Jersey	Insulin-dependent diabetes, end-stage renal disease (receiving dialysis), coronary artery disease (receipt of coronary artery bypass graft), GI bleeding, and polyp removal with transfusion	Nausea, cough, vomiting, weakness, and fever	Low platelet count on CBC with 8% *Babesia* species found by manual PB smear; confirmed by PCR as *B. microti*; atovaquone, clindamycin, and quinine failed to prevent respiratory failure, hypotension, cardiac complications, and progressive shock	Resident of New Jersey; *B. microti* IFA titer of 1:128
8	61	Female	Indiana	End-stage renal disease (receipt of hemodialysis), congestive heart failure, GI bleed requiring transfusion at previous hospital admission	Nausea with fever while receiving hemodialysis	Initially treated for bacterial sepsis (vancomycin and ceftazidime), then parasitemia was treated with exchange transfusion; originally received a misdiagnosis of malaria; treated with clindamycin and quinidine; developed altered mental status and disseminated intravascular coagulation and died; positive PCR results and an IgG titer of 1:2048 for *B. microti*	Resident of Indiana; traveled to wooded areas of Wisconsin 2 months before donating blood; no known tick bite; IgG titer of >1:256 and IgM titer of 1:20 for *B. microti*; negative PCR result after donation
9	43	Female	Delaware	Congenital hypoplastic anemia (Diamond-Blackfan syndrome), splenectomy, hepatitis C virus infection, pulmonary hypertension, iron overload, and multiple transfusions	Fatigue, "shakes" for 4 days without fever, decreased appetite and loose bowel movements for 1 week, chronic dry cough with infiltrates on chest radiograph	Treated for pneumonia with levaquin, vancomycin, and oseltamivir; previous PB smear reexamined as positive for *Babesia* species treated with clindamycin and quinine and transferred to ICU because of respiratory distress	Resident of New Jersey; traveled to Rhode Island but no known tick bite; *B. microti*; IFA titer of 1:1024; negative PCR result; donated RBC unit was frozen and deglycerolized before transfusion

NOTE. The information in this table was reported through a passive surveillance system; we report here the information provided. BUN, blood urea nitrogen; CBC, complete blood count; GI, gastrointestinal; ICU, intensive care unit; IFA, indirect immunofluorescence antibody assay; PB, peripheral blood.

Table 3. Timing of clinical events in fatal cases involving transfusion-transmitted *Babesia* infection reported to the US Food and Drug Administration.

Timing	Patient								
	1	2	3	4	5	6	7	8	9
Date of implicated blood unit transfusion	9 April 1998	16 November 2005	6 December 2005	24 August 2006	20 September 2006	6 September 2006	17 September 2007	20 September 2007	26 November 2007
Latency period,[a] days	35	38	50	30	18	30	29	43	41
Time to diagnosis,[b] days	43	42	50	34	19	36	31	57[c]	45
Time to death, days	49	55	57	42	26	41	34	50	47

[a] Periods from the date of implicated transfusion to the onset of symptoms are approximate (based on available estimated dates of symptom onset).
[b] Posttransfusion diagnosis of *Babesia* infection.
[c] The patient died prior to diagnosis of *Babesia* infection.

Babesia infection should be considered among potential etiologies for otherwise unexplained fever in patients who have recently received blood products. Because of the mobility of donors and transportation of blood products, babesiosis should be considered even beyond geographical regions with naturally occurring disease. As noted in table 2, several donors did not live in areas of endemicity but had traveled to these regions before donating blood.

Patients presented with symptoms (table 2) that were typical of natural infections. Most developed altered mental status, renal failure, or respiratory distress. The interval from blood transfusion to symptom onset was 2.5–7 weeks (table 3). An earlier article reported a 1–9-week time frame for transfusion-transmitted babesiosis [17]. These ranges of latency periods contrast with the natural infection incubation time of 2–4 weeks.

With 1 exception, all patients received transfusions from August through December, consistent with the seasonality of *Babesia* infection. Chronic parasitemia in the donor may have accounted for the 1 case involving a blood transfusion in April.

Implicated donations were identified in all cases; the donors tested positive by peripheral blood smear or immunofluorescence antibody assay. Four donors' samples also tested by PCR had negative results. They may have been convalescent and no longer parasitemic or were PCR negative because of the small sampling volume. All donors were asymptomatic at donation and remembered no tick bite.

Because many babesiosis cases may escape recognition, questioning donors has limited preventive value [17]. *Babesia* species can survive blood banking procedures, including refrigeration, leukoreduction, and filtration; pathogen transmission through transfusion of RBCs, deglycerolized RBCs, or platelets can occur [1, 18–21]. *Babesia* parasites can survive in frozen RBCs, because the glycerol treatment prevents lysis.

In view of the short periods between symptom onset and death (5–17 days) (table 3), examination of a peripheral blood smear (or other testing, depending on availability and the level of clinical suspicion) for possible *Babesia* species should be considered early in the evaluation of unexplained fever during the first few weeks after transfusions, particularly in aspleni or otherwise immunocompromised patients. Infectious disease consultation may be required to microscopically distinguish *Babesia* species from *Plasmodium* organisms.

Although babesiosis is not nationally notifiable, reporting transfusion-transmitted *Babesia* infections to public health authorities can allow investigators to identify infected donors and interdict remaining units. Investigation of prior donations also allows testing of associated recipients.

Similarly, blood collectors should immediately report postdonation babesiosis to the transfusion facilities to expedite

Figure 1. Summary of babesiosis-related Biological Product Deviation Reports (BPDRs) received by the US Food and Drug Administration (FDA) during fiscal years 1997–2007 (the FDA fiscal year is from 1 October through 31 September). These data do not include reports of infected donors identified prospectively through antibody assay research trials. BPDRs may include >1 recipient unit or donation. Potential implication in transfusion-transmitted disease refers to reports that indicate the safety of a blood component unit that may have been affected (e.g., instances when a blood transfusion recipient received a diagnosis of babesiosis, but the donor could not be contacted for confirmation). Postdonation illness refers to illness in donors who notified the blood collection establishment after donation that they had received a diagnosis of babesiosis. Whether these donors were infected at the time of donation was unknown; all units (not yet transfused) from these donors were withdrawn, and the donors were indefinitely deferred.

prompt withdrawal of potentially infected unexpired blood components. We remind blood establishments of the requirement to submit fatality and BPDRs to the FDA.

Our data cannot distinguish whether the increase in the numbers of deaths and reports to the BPDR system reflect an increasing incidence of babesiosis and/or improved diagnosis and reporting. State Health Departments (e.g., in New York and Connecticut) have also seen an increase in the number of babesiosis case reports over the past 10 years [22–25].

Each year, >5 million recipients receive >14 million transfusions of whole blood or RBCs [26]. Transfusion-transmitted babesiosis appears to be rare, but increased clinician awareness of the possibility of babesiosis in febrile transfusion recipients may facilitate earlier diagnosis and more successful treatment. It will also trigger timely public health investigations to interdict extant infected units and alert other associated recipients, protecting others from this potentially fatal blood-borne pathogen.

Addendum. During final revisions of this article in late September 2008, the FDA received a report of another death associated with transfusion-transmitted babesiosis. An elderly woman in Minnesota died ~3 weeks after receipt of 2 units of RBCs. One of the donated units' retention segments was positive for *Babesia* species by serologic testing and PCR.

Acknowledgments

We thank Susan L. Cannon and Sharon L. O'Callaghan, for providing facility and Biological Products Deviations Reporting data; Joseph M. Tonning, for initial MedWatch assessment; and Sanjai Kumar, Toby A. Silverman, M. Miles Braun, Barbara L. Herwaldt, and the anonymous reviewers, for their reviews of an advanced draft of this work.

Potential conflicts of interest. All authors: no conflicts.

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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
				2008. 11. 20	該当なし	
一般的名称	新鮮凍結人血漿		研究報告の公表状況	Seed C, Kee G, Ismay S, Wong T, Keller A.. AABB Annual Meeting and TXPO 2008; 2008 Oct 4–7; Montreal.	公表国 オーストラリア	
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)					

研究報告の概要

○マラリア抗体検査-輸血伝播マラリア(TTM)のリスクを最小に抑える安全かつ有効な戦略

背景:マラリアのスクリーニングに関して、オーストラリア赤十字(ARCBS)は2005年7月から、従来の医療歴、渡航歴の収集から、リスクのある供血者に対し、リスクへの暴露を特定したときから最低4ヶ月間のマラリア抗体の検査を実施する代替戦略を導入した。

方法:マラリアに罹患後回復した、あるいは過去3年間にマラリア流行国へ渡航・居住した供血者の血液を、市販のマラリア抗体EIAを用いて検査した。陰性血液は輸血用として供給され、供血者は再度供血可能とされた。EIA反復陽性(RR)の血液は追加検査(リアルタイムプラスモジウムPCR及び 免疫抗原クロマトグラフィー)に供された。追加検査陰性の供血者は現在の感染を示す証拠がない「抗体陽性」と見なされた。追加検査で陽性となった供血者は感染の可能性があると見なされ、直ちに臨床診断に紹介された。

結果:2005年7月～2008年2月に合計122,713の供血血液のEIA検査が実施され、そのうち117,900(96.1%)は陰性であり、ARCBSは159,287本のRBCおよび17,815本の血小板を供給した。EIA RR 4,813(3.9%)のうち1例で、PCRによる低レベルのプラスモジウムDNA が検出された(初回検体31、追加検体50copies/mL)が、抗原は陰性であった。この供血者はリベリア移民で幼少時にマラリアの既往歴があったが、追跡調査時には症状はなかった。

結論:この検査戦略の開始以降、既存の供血者に由来する輸血可能製剤の製造効率は著しく向上し、TTM症例の報告もなかった。

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」

血液を介するウイルス、細菌、原虫等の感染
vCJD等の伝播のリスク

報告企業の意見	今後の対応
オーストラリア赤十字(ARCBS)は2005年7月から、マラリア感染のリスクがある供血者に対し、リスクへの暴露を特定したときから最低4ヶ月間のマラリア抗体のスクリーニングを実施する代替戦略を導入した結果、既存の供血者に由来する輸血可能な製剤の製造効率が著しく向上し、輸血伝播マラリア症例の報告もないとの報告である。	日本赤十字社では、輸血感染症対策として問診時に海外渡航歴の有無を確認し、帰国(入国)後4週間は献血不適としている。また、マラリア流行地への旅行者または居住経験者の献血を一定期間延期している(1～3年の延期を行うとともに、帰国(入国)後マラリアを思わせる症状があった場合は、感染が否定されるまでの間についても献血を見合わせる)。今後も引き続き、マラリア感染に関する新たな知見及び情報の収集、対応に努める。

these findings, and showed both haplotypes have altered h^a and h^b antigen expression, and the associated c and e antigens are altered to the extent that alloanti-c and alloanti-e were produced. We also describe a homozygous proband with an alloantibody to the high-prevalence antigen that is antithetical to JAL. **Methods:** Samples from 15 JAL+ persons (11 probands and 4 family members including two Caucasian, six African American Blacks, one Puerto Rican Black, and six Brazilian Blacks) were tested. Hemagglutination studies were performed by standard methods using reagents from multiple sources. **Results:** The JAL+ status of the RBCs was determined with three anti-JAL (J Pas., Allen, MacD). RBCs from both Caucasian JAL+ probands had the (C)(e) haplotype and altered C, e, h^a, and h^b antigens. RBCs from Black JAL+ persons had the (c)(e) haplotype and expressed altered c, e, f, V, h^a, VS, and h^b antigens. Of six anti-C reagents, all agglutinated the two Caucasian samples moderately. Of nine anti-c reagents tested with RBCs from the non-Caucasian probands, reactivity ranged from strong to negative, notably, clone MS42 reacted strongly, MS33 and BS240 reacted moderately, and Gamma clone 951 was non-reactive. Of 12 anti-e reagents, reactivity ranged from strong to negative; notably, clone MS62 and MS63 reacted moderately, MS69 and HIRO36, 41, and 43 reacted weakly to moderately, and MS16 (depending on the formulation) was weakly reactive or non-reactive. All RBC samples were tested with at least two examples of anti-V, -h^a, -VS, and -h^b; some gave weak reactions while others did not react. Plasma from one proband contained alloanti-c, from another contained alloanti-e, and from a third contained an alloantibody that failed to react with D- - and Rh_{mod} RBCs. This apparent anti-Rh17 recognizes the high-prevalence antigen antithetical to JAL that we have named CES7. **Conclusions:** The presence of the JAL antigen has a quantitative and qualitative effect on the expression of, in Caucasians C, e, h^a, and h^b antigens, and in people of Black African ancestry c, e, f, V, h^a, VS, and h^b antigens. The qualitative effect on two antigens is revealed by two patients who received blood transfusions and made alloanti-c or alloanti-e. This is the first description of an antibody to the high-prevalence antigen antithetical to JAL.

Disclosure of Conflict of Interest
Christine Lomas-Francis, Connie Westhoff, Denden Alcantara, Pamela Nickle, Joan Uehlinger: Nothing to Disclose
Marion E. Reid: Not Specified

S36-020D
The JAL Antigen (RH46) is Encoded by an ARG114TRP Mutation
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Background: Two JAL-positive (Rh46+) haplotypes have been described. One, found in Caucasians, has altered C and e antigens ((C)(e)), and the other, found in people of African Black ancestry, has altered c and e antigens ((c)(e)) (Lomas, et al., Vox Sang 1990;59:39). The purpose of this study was to determine the molecular basis associated with expression of the JAL antigen. **Methods:** Samples from fifteen JAL+ probands; two Caucasian, six African American Blacks, six Brazilian Blacks, and one Puerto Rican were included in the study. Hemagglutination studies were performed by standard methods. DNA, extracted from peripheral blood leukocytes, was amplified by PCR and analyzed by sequencing. Reticulocyte RNA isolation and Rh-cDNA analysis was performed by standard molecular methods. **Results:** Samples from Caucasian JAL+ probands had RHCE*Ce, and those from Black JAL+ probands had RHCE*ce, but all had a 340C > T (Arg114Trp) missense mutation in exon 3 of RHCE regardless of the Ce or ce background allele. The JAL-encoding RHCE*ce allele also had 733C > G (Leu245Val) and red cells from informative heterozygotes and homozygotes did not express f, hr^a, or h^a antigens, and had dramatically weakened V and VS antigens. Expression of c antigen was also altered, as the RBCs were non-reactive with one of three monoclonal anti-c tested. In kind, expression of C on the Ce allele was altered, as was the e antigen expressed from both the ce or Ce allele that encoded JAL. **Conclusions:** Expression of the JAL (RH46) antigen results from 340C > T (Arg114Trp) on either a Ce or ce background. Although the Ce allele encoding the JAL antigen has been reported as CeMA by Nolzat-Pirenne et al., (Transfusion 2002;42:627) and the ce allele as ceS(340) by the same authors (Blood 2002; 100:4223), these alleles have not previously been recognized as encoding the JAL antigen. Those samples were studied because of altered expression of C or e antigen, respectively. We show here for the first time that, in addition to altered expression of C or c and e, the 114Trp mutation encodes the low-prevalence Rh antigen, JAL.

Disclosure of Conflict of Interest
Connie Westhoff, Sunitha Vege, Christine Lomas-Francis, Kim Hue-Roye, Lilian Castilho, Marion E. Reid: Nothing to Disclose

Transfusion Transmitted Diseases: Malaria and Chagas Disease

S37-020E
Malaria Antibody Testing—A Safe and Efficient Strategy to Minimize the Risk of Transfusion Transmitted Malaria (TTM)
C Seed, (akeiler@arcbs.redcross.org.au), G Kiel, S Ismay, T Wong, A Keiler, Australian Red Cross Blood Service, Perth, Australia; Sydney, Australia.

Background: Until recently, owing to the lack of a suitable test, screening for malaria in Australian blood donors involved collecting a medical and travel history and excluding the 'cellular' blood components from donors with a potential malaria exposure. This strategy minimised the residual risk of TTM to less than 1 in 10 million but resulted in the unavailability for transfusion of approximately 35,000 red blood cells (RBC) per annum. In July 2005 the Australian Red Cross Blood Service (ARCBS) implemented an alternative strategy based on screening donors with potential malaria exposure for malaria antibodies a minimum of 4 months after their last identified risk exposure. **Methods:** Since July 2005, donations from donors identified as having had malaria and recovered, or having travelled to or resided in a malarial country within the last 3 years are eligible for testing. RBC from these donors were collected, quarantined and samples tested using a commercial malaria antibody EIA. Non reactive (NR) donations were then issued for transfusion and the associated donors re-instated for cellular component manufacture. EIA repeatedly reactive (RR) donations were not issued and subject to supplementary testing (real-time plasmodial PCR and antigen immuno-chromatographic test). Concordantly supplemental test negative donors were considered 'antibody positive' without evidence of current infection. Donors reactive on either or both supplemental tests were considered potentially infected and referred immediately for clinical assessment. **Results:** A total of 122,713 donations were tested by EIA between July 2005 and February 2008, of which 117,900 (96.1%) were NR and the remaining 4,813 (3.9%) repeat reactive (RR). From donors re-instated by a NR EIA, ARCBS issued for transfusion an additional 159,287 RBC and 17,815 platelets representing the combined return from their tested donation, and subsequent donations made during the period of their original restriction. Of the 4,813 EIA RR samples, one had low level but reproducibly detectable plasmodial DNA by PCR (index sample 31 and follow-up sample 50 copies/uL) but was antigen negative. The donor, a migrant from Liberia had a history of malaria during childhood but was asymptomatic at follow up. **Conclusions:** The testing strategy has delivered in excess of 50,000 additional RBC and 5,000 additional platelets annually since inception. This has markedly improved manufacturing efficiency by increasing the proportion of transfusible components from existing donors. The lack of a reported TTM case since implementing the new strategy suggests that this dividend has been achieved without measurably impacting recipient safety.

Disclosure of Conflict of Interest
Clive Seed, Glenda Kee, Susan Ismay, Timothy Wong, Anthony Keiler: Nothing to Disclose

S38-020E
Malaria Deferrals: Time to Lessen the Impact of Travel Deferrals
M Nguyen, (gibble@usa.redcross.org), D Leiby, T Goff, J Gibble, American Red Cross, Rockville, MD; American Red Cross, Baltimore, MD; American Red Cross, Blood Services, Baltimore, MD.

Background: Human malaria, caused by five species of the intraerythrocytic protozoan parasite Plasmodium, remains a blood safety concern. However, blood banks are adversely impacted by ongoing donor loss due to an increasing number of malaria deferrals. Among the 1% of donors lost because of malaria deferrals, the majority (91%) were associated with travel to malaria endemic areas. In contrast, the only 2 transfusion-transmitted malaria cases reported in the US since 1998 were attributed to donors with past residence in an endemic region. This study investigated the relationship

別紙様式第2-1

No. 11

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. 2. 18	該当なし	
一般的名称	新鮮凍結人血漿		研究報告の公表状況	Am J Trop Med Hyg. 2009 Feb;80(2):215-7. Lee KS, Kim TH, Kim ES, Lim HS, Yeom JS, Jun G, Park JW.	公表国 韓国	
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)					

研究報告の概要

○韓国におけるクロロキン耐性三日熱マラリア
韓国において、三日熱マラリア(*Plasmodium vivax* malaria)が再興した1993年以降の患者は約100万人と推定される。この状況に対処するため、1997年より韓国軍はヒドロキシクロロキンおよびプリマキンを用いた予防的化学療法を実施している。予防的化学療法を受けた韓国軍兵士の累積者数は、2007年までに140万人を超えた。広範な予防化学療法を実施することで、韓国軍におけるマラリア患者の急増を防ぐことができたが、クロロキン(CQ)耐性*P. vivax*株発現の可能性が高まった。
本調査では、2003～2007年の期間に、韓国の*P. vivax*マラリア患者の治療効果のモニタリングを行い、調査登録患者484名中2名にCQ耐性を確認した。本結果は、アジア温帯地域におけるCQ耐性*P. vivax*の初めての報告である。韓国における*P. vivax*のCQ耐性発現頻度の変動をモニターするには、継続的調査が必要である。

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」

血液を介するウイルス、細菌、原虫等の感染
vCJD等の伝播のリスク

報告企業の意見	今後の対応
1997年より韓国軍はヒドロキシクロロキンおよびプリマキンを用いた予防的化学療法を実施し、マラリア患者の急増を防ぐことができたが、調査登録患者484名中2名にクロロキン(CQ)耐性*Plasmodium vivax*を確認したとの報告である。	日本赤十字社では、輸血感染症対策として問診時に海外渡航歴の有無を確認し、帰国(入国)後4週間は献血不適としている。また、マラリア流行地への旅行者または居住経験者の献血を一定期間延期している(1～3年の延期を行うとともに、帰国(入国)後マラリアを思わせる症状があった場合は、感染が否定されるまでの間についても献血を見合わせる)。今後も引き続き、マラリア感染に関する新たな知見及び情報の収集、対応に努める。

Am. J. Trop. Med. Hyg., 80(2), 2009, pp. 215–217

Short Report: Chloroquine-resistant *Plasmodium vivax* in the Republic of Korea

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Abstract. The number of *Plasmodium vivax* malaria patients in the Republic of Korea and North Korea since the re-emergence of malaria in 1993 is estimated to be approximately one million. To cope with this situation, the Army of the Republic of Korea has performed chemoprophylaxis with hydroxychloroquine and primaquine since 1997. The cumulative number of soldiers in the Army of the Republic of Korea given chemoprophylaxis exceeded 1.4 million by 2007. Extensive chemoprophylaxis contributed to preventing a rapid increase of malaria patients in the Army of the Republic of Korea, but increased the possibility of the occurrence of chloroquine (CQ)–resistant *P. vivax* strains. In this study, treatment responses of *P. vivax* malaria patients in the Republic of Korea monitored during 2003–2007, and CQ resistance was confirmed in 2 of 484 enrolled patients. Our results are the first report of CQ-resistant *P. vivax* in a temperate region of Asia. Continuous surveillance is warranted to monitor the change in CQ resistance frequency of *P. vivax* in the Republic of Korea.

Plasmodium vivax malaria, which was endemic on the Korean Peninsula for many centuries until the late 1970s, re-emerged in 1993 in the Republic of Korea.[1] The malaria-prevalent area has been confined to the area adjacent to the Demilitarized Zone (DMZ) from the early stage of the re-emergence, and malaria occurrence in the Republic of Korea has been directly influenced by the prevalence of malaria in the region of North Korea located near the DMZ.[1–3] The total number of malaria patients in the Republic of Korea and North Korea since the re-emergence likely approaches one million.[1–4] To cope with the situation, the Army of the Republic of Korea has performed chemoprophylaxis with hydroxychloroquine (HCQ) and presumptive anti-relapse therapy with primaquine since 1997.[5] The cumulative number of the soldiers in the Army of the Republic of Korea given chemoprophylaxis exceeded 1.4 million by 2007. This extensive chemoprophylaxis campaign has helped prevent a rapid increase of malaria patients in the Army of the Republic of Korea. However, this success is tempered by the increased possibility of chloroquine (CQ)-resistant *P. vivax* strains.[5]

In this study, 484 patients from 6 hospitals in the Republic of Korea (5 in the malaria-prevalent region and 1 in Seoul) were enrolled during 2003–2007. Blood samples were collected from all patients before HCQ treatment and 24 hours after completion of treatment. Treatment responses were monitored by investigation of fever clearance time and parasite clearance time. Plasma concentrations of HCQ before and 24 hours after completion of treatment were measured by validated reversed-phase high-performance liquid chromatography[6] with slight modifications.[7] Additional examinations or blood collection were not performed. The study protocols were reviewed and approved by the institutional review board of each hospital. All patients enrolled in this study were admitted to the hospitals during HCQ treatment, and HCQ was taken under the physician supervision. There were no problems with HCQ treatment compliance.

Among 484 patients enrolled in the five-year study, HCQ treatment failed in two patients (Table 1). These two patients had not been in malaria-prevalent areas in other nations during the two years prior to their present hospitalization.

Patient A was a 26-year-old man (civilian) who had been discharged from the military in May 1998. Chemoprophylaxis was not performed during his military service. He was admitted to hospital I located in Goyang, a malaria-prevalent area in Kyonggi Province, on July 30, 2003. *Plasmodium vivax* malaria was confirmed and he was administered 2,000 mg of HCQ over a three-day period. More specifically, on day 0, he was given 800 mg of HCQ, with doses of 400 mg administered 6 hours and 24 hours later (day 1), and 48 hours later (day 2). Despite administration of the first cycle of HCQ treatment, fever did not subside until day 6 and *P. vivax* trophozoites were evident in a peripheral blood smear obtained on day 6. Parasite density on day 0 (before the treatment) and day 3 (24 h after completion of HCQ treatment) were 3,500/μL and 300/μL, respectively. Gene amplification by species-specific primers for small subunit ribosomal RNA[8] showed that *Plasmodia* in the patient's peripheral blood was *P. vivax*. The plasma concentration of HCQ 24 hours after the completion of HCQ treatment was 165 ng/mL. The patient was completely cured by administration of an additional cycle of HCQ treatment commencing on day 6.

Patient B was a 72-year-old woman. She was admitted to hospital II located in Seoul on July 24, 2007 (day 0), because of fever and chills. *Plasmodium vivax* malaria was diagnosed and HCQ was administered on July 25–27 (days 1–3). Treatment was unsuccessful in resolving the fever and severe headache, and parasites were evident both microscopically and by small subunit ribosomal RNA amplification until day 4. Parasite density on days 0 and 4 was 3,800/μL and 440/μL, respectively. The plasma concentration of HCQ 24 hours after the completion of HCQ treatment was 150 ng/mL. Salvage treatment with quinine sulfate and doxycycline was carried out for seven days beginning on day 4, followed by administration of primaquine. This regimen completely resolved the infection.

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TABLE 1
Demographic and clinical characteristics of two patients unsuccessfully treated with the conventional HCQ regimen, Republic of Korea*

Patient	Hospital (location)	Period of HCQ administration	Plasma concentration of HCQ† (ng/mL)	Parasite density before/after HCQ treatment (parasites/μL)	Regimen for complete cure
A	I (Goyang)	July 30–August 1, 2003	165	3,500/300	Additional administration of HCQ
B	II (Seoul)	July 25–27, 2007	150	3,800/440	Quinine sulfate and doxycycline

* HCQ = hydroxychloroquine.
† Measured 24 hours after completion of HCQ treatment.

Chloroquine-resistant *P. vivax* strains have been reported from various areas[9–12] since its emergence in Papua New Guinea in 1989.[13] In the Republic of Korea, a large-scale chemoprophylaxis campaign has been performed since 1997. However, prophylaxis has consistently failed in many cases despite attainment of sufficiently high plasma concentrations of HCQ. Moreover, the length of time required for the elimination of *P. vivax* from patients' blood by HCQ treatment has increased in the current decade.[14]

Hydroxychloroquine has been reported to be as active as CQ against malaria parasites,[15,16] and 400 mg of HCQ is the molar equivalent of 309.6 mg of HCQ base and 295.0 mg of CQ base. Therefore, a CQ concentration of 10 ng/mL in plasma, which is the minimum effective concentration against CQ-susceptible *P. vivax*, is equivalent to an HCQ concentration of 10.5 ng/mL of plasma. In this study, treatment with 2,000 mg of HCQ over a three-day period was not effective in 2 (0.4%) of 484 patients. For these two patients, plasma concentrations of HCQ 24 hours after completion of HCQ treatments were much higher than the minimum effective concentration of CQ against *P. vivax*.[17] For the 482 patients with successful therapeutic outcomes, the mean and the standard deviation of plasma concentrations of HCQ 24 hours after completion of HCQ treatments were 220 ng/mL and 121 ng/mL, respectively, which were in not distinct from the two patients in whom HCQ treatment failed. This indicates that HCQ was absorbed and metabolized normally in the two patients, precluding the possibility that the treatment failure was caused by personal factors. In the two patients, parasitemias were reduced markedly, but not cleared, by HCQ administration. Patient A was cured by additional administration of HCQ; this success may have been the result of the infecting *P. vivax* being exposed to an increased trough concentration of HCQ for an extended period because of the cumulative dosage.

The present observations are the first report of CQ-resistant *P. vivax* from a temperate region of Asia. Surveillance activity should be strengthened to monitor the change of CQ susceptibility of *P. vivax* in the Republic of Korea.

Received August 14, 2008. Accepted for publication November 1, 2008.

Acknowledgments: We thank Young-A Kim, Sun-Young Ahn, Mi-Young Shin, and Ji-Ae Yoo for technical support.

Financial support: This study was supported by a grant from the Korea Health 21 Research and Development project, the Ministry of Health and Welfare, the Republic of Korea (A030075), and a grant from the Korea Centers for Disease Control and Prevention, the Republic of Korea (no. 2008-E00139-00).

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別紙様式第2−1

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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 2009年3月13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	Simian Malaria in a U.S. Traveler --- New York, 2008	公表国 米国	
販売名(企業名)	別紙のとおり					

研究報告の概要	使用上の注意記載状況・その他参考事項等
問題点：サルマラリアである*Plasmodium knowlesi*のヒトへの感染例がマレーシアおよびその周辺の広範囲において多数報告され、人畜共通感染症の病原体として新興している可能性が示されている。 4種のプラスモディウム属の赤血球内原虫（熱帯熱マラリア原虫；*P. falciparum*、三日熱マラリア原虫；*P. vivax*、四日熱マラリア原虫；*P. malaride*および卵形マラリア原虫；*P. ovale*）がヒトでマラリアを起こすことが知られている。しかし、最近のアジアからのレポートで、5番目のマラリア原虫として*Plasmodium knowlesi*が人畜共通感染症の病原体として新興している可能性が示されている。20種類以上のマラリア原虫がヒト以外の霊長類に感染するが、これまでサルマラリアのヒトへの自然感染は、公衆衛生学に重要でない稀な事象とされてきた。光学顕微鏡による観察では、多くのサルマラリア原虫はヒトにマラリアを起こす4種のマラリア原虫との鑑別はほぼ困難で、PCRやマイクロサテライト分析といった分子的技術が種の確定に必要である。 最初の*P. knowlesi*感染は、1965年に東南アジアの任務から戻ってきた米国の兵士であった。その後の報告はほとんどなく、2002年にマレーシアの研究者らが非典型的な特徴をもつ四日熱マラリア症例の増加や、より重篤な臨床症状、より高度な寄生虫血症に気付いている。nested PCR assayにより、これらのマラリア症例の50%以上が*P. knowlesi*であると確認された。最初に顕微鏡診断されていた四日熱マラリアは1例もなかった。2001〜2006年に同じ研究者らによって行われたレトロスペクティブな調査では、マレーシアのSarawak州の患者からの960検体のうち28%が*P. knowlesi*であった。以前は、そのほとんどが形態学的に四日熱マラリアと診断されていた。このグループはまた、四日熱マラリアによる重症のマラリアと考えられていた4例の異常な死亡が、後にPCRによって*P. knowlesi*と確認されたことも報告している。さらにヒトの*P. knowlesi*感染は、シンガポール、タイとミャンマーの国境、フィリピン、中国の雲南省、フィンランド（マレーシアから帰った旅行者が最初熱帯熱マラリアと誤診されていた）からも報告されている。	記載なし

報告企業の意見	今後の対応
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。

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別紙

一般的名称	①人血清アルブミン、②人血清アルブミン、③人血清アルブミン*、④人免役グロブリン、⑤乾燥ペプシン処理人免疫グロブリン、⑥乾燥スルホ化人免疫グロブリン、⑦乾燥スルホ化人免疫グロブリン*、⑧乾燥濃縮人活性化プロテインC、⑨乾燥濃縮人血液凝固第Ⅷ因子、⑩乾燥濃縮人血液凝固第Ⅸ因子、⑪乾燥抗破傷風人免疫グロブリン、⑫抗HBs人免疫グロブリン、⑬トロンビン、⑭フィブリノゲン加第ⅩⅢ因子、⑮乾燥濃縮人アンチトロンビンⅢ、⑯ヒスタミン加人免疫グロブリン製剤、⑰人血清アルブミン*、⑱人血清アルブミン*、⑲乾燥ペプシン処理人免役グロブリン*、⑳乾燥人血液凝固第Ⅸ因子複合体*、㉑乾燥濃縮人アンチトロンビンⅢ
販売名(企業名)	①献血アルブミン20“化血研”、②献血アルブミン25“化血研”、③人血清アルブミン“化血研”*、④“化血研”ガンマーグロブリン、⑤献血静注グロブリン“化血研”、⑥献血ベニロンーⅠ、⑦ベニロン*、⑧注射用アナクトC2,500単位、⑨コンファクトF、⑩ノバクトM、⑪テタノセーラ、⑫ヘパトセーラ、⑬トロンビン“化血研”、⑭ボルヒール、⑮アンスロビンP、⑯ヒスタグロビン、⑰アルブミン20%化血研*、⑱アルブミン5%化血研*、⑲静注グロブリン*、⑳ノバクトF*、㉑アンスロビンP1500注射用
報告企業の意見	マラリアは、ハマダラ蚊によって媒介されるが、ヒトに感染すると赤血球に侵入し、増殖した後、赤血球を破壊し次の赤血球に侵入するサイクルを繰り返す。このような生活環から、稀ではあるが輸血によるマラリア感染も報告されている。 仮に、本剤の原材料であるヒト血液にマラリア原虫が混入していたとしても、当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、マラリア原虫よりも小さいウイルスの除去を目的とした平均孔径19nm以下の「ウイルス除去膜ろ過工程」が導入されているので、これらの工程により除去されるものと考えられる。更に、これまでに本剤によるマラリアの報告例は無い。以上の点から、本剤はマラリアに対して一定の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

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March 13, 2009 / 58(09);229-232

Simian Malaria in a U.S. Traveler --- New York, 2008

Four species of intraerythrocytic protozoa of the genus *Plasmodium* (*P. falciparum, P. vivax, P. ovale*, and *P. malariae*) are known to cause malaria in humans. However, recent reports from Asia suggest the possibility that a fifth malaria species, *Plasmodium knowlesi*, is emerging as an important zoonotic human pathogen. Although more than 20 species of *Plasmodium* can infect nonhuman primates, until recently, naturally acquired human infections of simian malaria were viewed as rare events lacking public health significance. When viewed by light microscopy (the gold standard for laboratory diagnosis of malaria), many of the simian species are almost indistinguishable from the four *Plasmodium* species that cause infection in humans (Table). Molecular techniques, such as polymerase chain reaction (PCR) amplification and microsatellite analysis, are needed for definitive species determination. This report describes the first recognized case of imported simian malaria in several decades in the United States, diagnosed in 2008 in a patient from New York who had traveled to the Philippines. Atypical features of the parasite seen on light microscopy triggered further molecular testing, which confirmed the diagnosis of *P. knowlesi*. To date, all simian malaria species have been susceptible to chloroquine treatment. Molecular analysis of certain malaria parasites isolated from ill travelers returning to the United States from Asia or South America can more accurately assess the burden of simian malaria parasite infections in humans.

The first recognized case of naturally acquired simian malaria was a 1965 case of *P. knowlesi* infection in an employee of the U.S. Army who had returned home from an assignment in Southeast Asia (*1*); subsequent reports were few and unconfirmed. In 2002, investigators in Malaysia noted an increasing number of *P. malariae* cases with atypical features, including increased clinical severity and higher parasitemia (*2*). By using a nested PCR assay, more than 50% of these malaria cases were determined to be *P. knowlesi*, none were *P. malariae*, as originally determined by microscopy (*2*). In a retrospective evaluation by the same investigators during 2001—2006, 28% of 960 specimens from patients in Sarawak, Malaysian Borneo, were found to be *P. knowlesi*, after being morphologically diagnosed most often as *P. malariae* (*3*). The group also reported four unusual fatalities attributed to severe malaria caused by *P. malariae* that was later confirmed as *P. knowlesi* by PCR. Additional cases of naturally occurring *P. knowlesi* infection in humans have been reported from Singapore (*4*), the Thai-Burma border (*5*), the Philippines (*6*), Yunnan Province in China (*7*), and Finland, where a returning traveler from Malaysia was misdiagnosed initially as having infection with *P. falciparum* (*8*).

Case Report

In the recent U.S. case, a woman aged 50 years with no previous history of malaria who was born in the Philippines but had lived in the United States for 25 years, returned to her home country to visit friends and relatives on October 17, 2008. While there, she stayed on the island of Palawan in a cabin located at the edge of a forested area known to be a habitat for long-tailed macaques. She had not taken malaria chemoprophylaxis and had not used any mosquito-avoidance measures, both of which are recommended preventive measures for travelers to this area.

The woman returned to the United States on October 30, 2008, and noted the onset of a headache. Fever and chills ensued, and symptoms persisted for several days, after which she sought medical attention. In the emergency department, she was noted to be hypotensive and to have thrombocytopenia. Examination of thick and thin malaria smears (Figure 1) was ordered, and an initial, erroneous diagnosis of babesiosis was made by a laboratory technician. Upon review by the laboratory supervisor the following morning, the diagnosis was reassessed as malaria with 2.9% of red cells parasitized. However, the atypical appearance of the *Plasmodium* sp. seen in the smears prevented a species-specific diagnosis. The woman was treated successfully with atovaquone-proguanil and primaquine for *Plasmodium* of undetermined species.

An ethylenediaminetetraacetic acid (EDTA) blood tube and two stained smears were sent to New York state's Wadsworth Center Parasitology Reference Laboratory for confirmation of species by PCR. The Wadsworth Center confirmed the presence of atypical rings and schizonts of a *Plasmodium* species (Figure 1), but conventional PCR targeting the small subunit (SSU) of rRNA did not yield a product consistent with any of the four species of *Plasmodium* known to infect humans. The specimen also was negative for the variants of *P. ovale*, which are commonly seen in Southeast Asia. However, primers specific for the SSU rDNA of the genus *Plasmodium* yielded a 1,055-bp PCR product that was sequenced and noted to be a 99% match over its full length to the SSU rRNA gene from *P. knowlesi* (H strain) (*9*). These data confirmed that the infection was caused by *P. knowlesi*.

Reported by: *JG Ennis, AE Teal, A Habura, PhD, S Madison-Antenucci, PhD, JS Keithly, PhD, Div of Infectious Diseases, New York State Dept of Health. PM Arguin, MD, JW Barnwell, PhD, WE Collins, PhD, S Maki, MPH, L Slutsker, MD, A Dasilva, DSc, Div of Parasitic Diseases, National Center for Zoonotic, Vector-Borne, and Enteric Diseases. J Hwang, MD, EIS Officer, CDC.*

Editorial Note:

Several conditions need to coincide for simian species of *Plasmodium* to infect humans: 1) human erythrocytes must be susceptible

to invasion by simian parasites, 2) humans must be near or in forests where nonhuman simians are infected, and 3) anopheline mosquitoes that feed on both humans and nonhuman simians must be present (*10*). Many areas in Asia and South America have overlapping populations of nonhuman primates that serve as reservoirs for simian malaria and competent *Anopheles* mosquito vectors that are necessary to transmit the infection to humans (Table, Figure 2) (*1*). For *P. knowlesi* in Asia, the normal hosts are long-tailed and pig-tailed macaques and mitered-leaf monkeys, which are found with *Anopheles* mosquito vectors of the Leucosphyrus group, enabling transmission of infection (*1*). Other simian malaria species known to infect humans include *P. simium* and *P. brasilianum* in South America and *P. cynomolgi* and *P. inui* in Asia (*1,10*).

Most simian malaria infections in humans can cause mild or moderate disease but often are self-limited, not requiring antimalarial therapy (*1*). However, *P. knowlesi*, with its 24-hour asexual replication cycle, can result in large parasite burden and severe, life-threatening disease (*3*). Severe malaria imported from Asia should alert the physician to the possibility of infection with *P. knowlesi*. Health-care providers also should consider hospitalization if the patient with malaria reports travel to forested areas of Asia, where *P. knowlesi* transmission occurs. Simian *Plasmodium* species are susceptible to all available antimalarials in the United States. Although definitive diagnosis as a simian species of *Plasmodium* cannot be made in time to guide selection of antimalarials at the initiation of therapy, treatment for undetermined *Plasmodium* species will effectively treat all simian species. Use of current treatment and chemoprophylaxis guidelines are appropriate for treating and preventing simian malaria infections in humans.

Health-care providers of patients with malaria and laboratories that diagnose malaria imported from Asia or non-falciparum malaria from South America should refer appropriate specimens to a Clinical Laboratory Improvement Amendments (CLIA)–verified state health reference laboratory or CDC's Division of Parasitic Diseases Reference Laboratory for species confirmation by molecular testing. In the United States, approximately 1,500 malaria cases are reported each year, almost all imported from areas where malaria is endemic; approximately 200 of these cases are imported from Asia or South America. In the United States, the potential for not recognizing a *Plasmodium* infection of simian origin is high because diagnosis usually relies on microscopic examination of Giemsa-stained smears rather than diagnosis by molecular techniques. Only a few laboratories (including state and federal public health reference and commercial laboratories) routinely use molecular assays, and even fewer have the capacity to confirm simian species.

The substantial number of recent human cases of simian malaria reported in Malaysia and the wider region (including the travel-associated case described in this report) underscores the need to define the scope and magnitude of the problem (*2—8*). Persons wishing to send specimens for species confirmation by CDC should collect pretreatment blood in EDTA or acid citrate dextrose blood collection tubes. Instructions and specimen submission forms are available online at http://www.cdc.gov/malaria/smscs.htm. Contact information for local or state health department laboratories is available at http://www.aphl.org/aboutaphl/aboutphls/pages/memberlabs.aspx. As with all suspected cases of malaria, health-care providers with questions regarding diagnosis or treatment should call the CDC Malaria Hotline at 770-488-7788 (Monday—Friday, 8:30 a.m. to 4:30 p.m., EST). Health-care providers seeking emergency consultation after hours should call 770-488-7100 and request to speak with a CDC Malaria Branch clinician.

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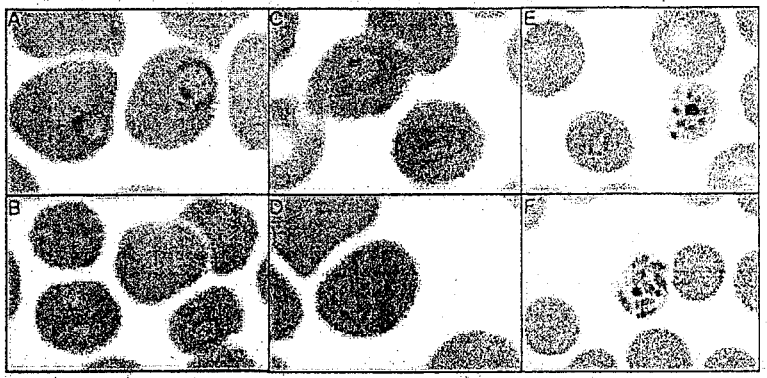
Figure 1

FIGURE 1. Giemsa-stained blood smears (1,000x magnification) from a reported case of *Plasmodium knowlesi* infection, highlighting the various features that often are mistaken for *Plasmodium malariae* or *Plasmodium falciparum — New York, 2008**



* Panel A. An infected red blood cell (RBC) with trophozoites resembling *P. malariae*. Panel B. Multiple infected RBCs, which are more commonly observed with *P. falciparum*. Panels C and D. Infected RBCs with "band-form" trophozoites resembling *P. malariae*. Panel E. RBC with eight merozoites in rosette-pattern resembling *P. malariae*. Panel F. *P. knowlesi* merozoites, although similar in appearance to *P. malariae*, are smaller and occupy less space in the infected RBC.

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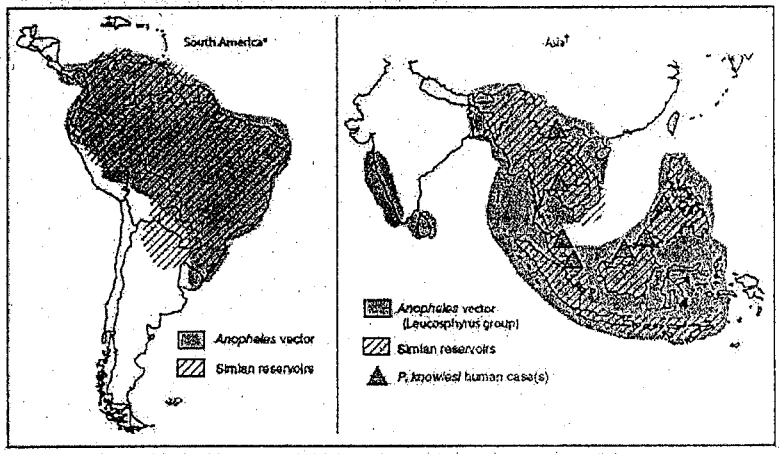
Figure 2

FIGURE 2. Overlapping distributions of competent *Anopheles* vectors and potential simian reservoirs for *Plasmodium brasilianum* and *Plasmodium simium* in South America and *Plasmodium knowlesi* in Asia



* Distribution of competent *Anopheles* and various simian reservoirs known to be infected with either *P. brasilianum* or *P. simium*.
† Distribution of *Anopheles* mosquitoes of the Leucosphyrus group and various simian reservoirs necessary for *P. knowlesi* human infection. Both single and clusters of human cases of *P. knowlesi* were reported from Malaysian Borneo, Peninsular Malaysia, China, Philippines, Singapore, and Thailand during 2001–2008.

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Table

TABLE. Simian malaria species in Asia and South America with their associated geographic distribution and morphologic similarity to one of four human *Plasmodium* species*

Simian *Plasmodium* species	Geography	Human species they resemble
Asia		
P. coatneyi	Malaysia, Philippines	*P. falciparum*
P. cynomolgi	India, Indonesia, Malaysia, Sri Lanka, Taiwan	*P. vivax*
P. eylesi	Malaysia	*P. vivax*
P. fieldi	Malaysia	*P. ovale*
P. fragile	India, Sri Lanka	*P. falciparum*
P. hylobati	Indonesia	*P. vivax*
P. inui	India, Indonesia, Malaysia, Philippines, Sri Lanka, Taiwan	*P. malariae*
P. jeffreyi	Indonesia, Malaysia	*P. vivax*
P. knowlesi	China, Indonesia, Malaysia, Philippines, Singapore, Thailand, Taiwan	*P. malariae, P. falciparum*
P. pithecl	Malaysia	*P. vivax*
P. simiovale	Sri Lanka	*P. ovale*
P. silvaticum	Malaysia	*P. vivax*
P. youngi	Malaysia	*P. vivax*
South America		
P. brasilianum	Brazil, Colombia, Mexico, Panama, Peru, Venezuela	*P. malariae*
P. simium	Brazil	*P. vivax*

*Four species of intraerythrocytic protozoa of the genus *Plasmodium* (*P. falciparum, P. vivax, P. ovale,* and *P. malariae*) are known to cause malaria in humans.

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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
				2008. 12. 15	該当なし	
一般的名称	新鮮凍結人血漿		研究報告の公表状況	Zhang L, Liu Y, Ni D, Li Q, Yu Y, Yu XJ, Wan K, Li D, Liang G, Jiang X, Jing H, Run J, Luan M, Fu X, Zhang J, Yang W, Wang Y, Dumler JS, Feng Z, Ren J, Xu J. JAMA. 2008 Nov 19;300(19):2263-70.	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)				中国	

研究報告の概要	○中国におけるヒト顆粒球アナプラズマ症の院内感染 背景：ヒト顆粒球アナプラズマ症(HGA)は、中国の新興ダニ媒介性リケッチア疾患である。劇症HGA患者との接触後に医療従事者およびその家族の感染集団を認めたため調査が行われた。 目的：安徽省における発熱性疾患の院内感染と思われる症例の感染源および伝播について検討すること。 デザイン、実施場所、および患者：発熱、出血により病院の隔離病棟へ入院し死亡した発端患者への接触後に発熱性疾患を生じ、接触が疑われた二次症例患者の抗*Anaplasma phagocytophilum*抗体、PCR測定、*A. phagocytophilum* DNA配列決定を実施した。 主な評価項目：血清学的またはPCRによりHGAの確証が得られた症例を非感染接触者と比較し、発病率、疾患相対リスク、発端患者への医療提供時の曝露についての潜在的リスクを定義した。 結果：2006年11月9日～17日の期間に、白血球減少、血小板減少を伴う発熱と血清アミノトランスフェラーゼ値上昇を発現した9名の患者が、末梢血中*A. phagocytophilum* DNAのPCRおよび*A. phagocytophilum*へのセロコンバージョンによりHGAと診断された。ダニに刺咬された患者はいなかった。9名の患者はいずれも、発端患者が死亡する直前の12時間以内に患者と接触し、その12時間の間に当該患者は大量出血があり、また気管内挿管治療を受けた。発病率は、50cm以内の接触者が32.1% vs 0% (P=0.04)、2時間以上の接触者が45% vs 0% (P=0.001)、血性分泌物への接触報告者が75% vs 0% (P<0.001)、発端患者の呼吸器分泌物への接触報告者が87.5% vs 0% (P=0.004)であった。 結論：中国におけるHGAの特定および血液や呼吸器分泌物への直接的な接触による院内HGA感染の可能性について報告する。	
報告企業の意見		今後の対応
劇症ヒト顆粒球アナプラズマ症(HGA)患者との接触後の医療従事者および家族の感染集団についてPCR等で調査した結果HGAと特定され、血液や呼吸器分泌物への直接的な接触による院内*A. phagocytophilum*感染が示唆されたとの報告である。		日本赤十字社では、輸血感染症対策として問診時に海外渡航歴の有無を確認し、帰国(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。

ORIGINAL CONTRIBUTION

Nosocomial Transmission of Human Granulocytic Anaplasmosis in China

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Context Human granulocytic anaplasmosis (HGA) is an emerging tick-borne disease in China. A cluster of cases among health care workers and family members following exposure to a patient with fulminant disease consistent with HGA prompted investigation.

Objective To investigate the origin and transmission of apparent nosocomial cases of febrile illness in the Anhui Province.

Design, Setting, and Patients After exposure to an index patient whose fatal illness was characterized by fever and hemorrhage at a primary care hospital and regional tertiary care hospital's isolation ward, secondary cases with febrile illness who were suspected of being exposed were tested for antibodies against *Anaplasma phagocytophilum* and by polymerase chain reaction (PCR) and DNA sequencing for *A phagocytophilum* DNA. Potential sources of exposure were investigated.

Main Outcome Measure Cases with serological or PCR evidence of HGA were compared with uninfected contacts to define the attack rate, relative risk of illness, and potential risks for exposure during the provision of care to the index patient.

Results In a regional hospital of Anhui Province, China, between November 9 and 17, 2006, a cluster of 9 febrile patients with leukopenia, thrombocytopenia, and elevated serum aminotransferase levels were diagnosed with HGA by PCR for *A phagocytophilum* DNA in peripheral blood and by seroconversion to *A phagocytophilum*. No patients had tick bites. All 9 patients had contact with the index patient within 12 hours of her death from suspected fatal HGA while she experienced extensive hemorrhage and underwent endotracheal intubation. The attack rate was 32.1% vs 0% ($P=.04$) among contacts exposed at 50 cm or closer, 45% vs 0% ($P=.001$) among those exposed for more than 2 hours, 75% vs 0% ($P<.001$) among those reporting contact with blood secretions, and 87.5% vs 0% ($P=.004$) among those reporting contact with respiratory secretions from the index patient.

Conclusion We report the identification of HGA in China and likely nosocomial transmission of HGA from direct contact with blood or respiratory secretions.

JAMA. 2008;300(19):2263-2270 www.jama.com

HUMAN GRANULOCYTIC ANAPLASMOSIS (HGA) is an emerging tick-borne infectious disease that was recognized in the United States in 1990[1] and in Europe in 1997.[2] The disease name was changed from human granulocytic ehrlichiosis to HGA in 2001 when the causative rickettsia was reclassified from the genus ehrlichia as *Anaplasma phagocytophilum*.[3] Although the clinical presentation of HGA is variable and although it may be difficult to diagnose, the annual number of infections reported in the United States since 1990 has steadily increased.[4,5] Seroepidemiological data suggest that infection rates in endemic areas are as high as 15% to 36%,[6-8] implying that the diagnosis is often missed or that infection is mild or asymptomatic. Because epidemiological, clinical, and microbiological information about HGA is limited, the disease is likely underrecognized and underreported worldwide.[7]

Despite the pathogen's global distribution, only a limited number of laboratory-confirmed cases have been reported from countries in Europe, where the median seroprevalence rate is 6.2%, similar to that in North America.[9] Serological and molecular evidence also suggests that human infection exists in Korea, Japan, and China.[10-14] Herein, we report the first cases of HGA acquired in China, as well as the unusual finding of nosocomial human-to-human transmission.

For editorial comment see p 2308.

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METHODS

Laboratory Diagnosis

Patients suspected of HGA exposure were tested for serum IgG to *A phagocytophilum* using the IgG IFA kit (Focus Diagnostics, Cypress, California), screening at a 1:64 dilution and titrating if reactive.[15] Nested polymerase chain reaction (PCR) using blood DNA (QIAamp DNA Mini Kit, QIAGEN, Hilden, Germany) was used to detect *A phagocytophilum* DNA with *Anaplasma* and *Ehrlichia* genus-common and *A phagocytophilum* species-specific *rrs* primers (16S rRNA gene),[16] and *A phagocytophilum* species-specific *groEL* primers.[17] An *A phagocytophilum rrs* plasmid and DNA from healthy people or distilled water were used as controls. Positive reactions were confirmed by direct sequencing. Polymerase chain reaction was conducted in 2 independent laboratories, the National Institute for Communicable Disease Control and Prevention in Beijing, and at the Anhui Province Center for Disease Prevention and Control in Hefei city. Each laboratory used its own primers, reagents, and patient blood DNA. All samples were tested concurrently with negative and no template controls (water) under the same conditions. Polymerase chain reaction samples from healthy people and negative controls consistently had negative results.

To exclude other infections, serological, antigen detection, and PCR diagnostic tests were conducted. These included tests on blood from the first 3 to 5 days after onset for reverse transcription (RT)–PCR of PCR for nucleic acids of Lassa fever virus, Ebola virus, Marburg virus, Hantaan virus, Junin virus, yellow fever virus, Crimean-Congo hemorrhagic fever virus, coxsackievirus, respiratory syncytial virus, adenovirus, *Mycoplasma pneumoniae*, *Chlamydia* species, *Ehrlichia* species, *Richettsia* species, and *Orientia tsutsugamushi*.

Tests were also conducted on oropharygeal swabs from the first 3 to 5 days after onset for influenza A virus antigens, and by PCR for influenza A viruses, influenza B virus, and influenza virus subtype H5 nucleic acids. Tests for acute-phase serum were conducted to detect IgM and IgG to severe acute respiratory syndrome virus, as well as to detect IgM or IgM plus IgG antibodies by capture enzyme-linked immunosorbent assay against Bunyaviridae, Filoviridae, Lassa fever virus, Ebola virus, Marburg virus, Hantaan virus, Junin virus, yellow fever virus, and Crimean-Congo hemorrhagic fever virus.

Epidemiological Investigation

All contacts of the index patient, including patients with similar clinical presentations and healthy persons, were interviewed before laboratory diagnostic results were obtained. A possible case of HGA was defined as a patient with a clinically compatible illness (fever, headache, chills) and laboratory findings including thrombocytopenia and leukopenia but who lacked serological or molecular tests for *A phagocytophilum*. A confirmed case was defined as a patient with a clinically compatible illness (as above) and in keeping with the US Centers for Disease Control and Prevention (CDC) criteria (http://www.cdc.gov/ncphi/disss/nndss/casedef/ehrlichiosis_2008.htm) by either seroconversion, a 4-fold increase in *A phagocytophilum* IgG antibody titer in acute and convalescent sera, or a positive PCR result for both *A phagocytophilum rrs* and *groEL* confirmed by direct sequence analysis.[15]

Contact Questionnaire

All contacts of the index patient were asked to complete a questionnaire about their health status and profession; experience with tick bites; exposure to the index patient—where, when, and how they had contact; exposure to wild animals; extent of outdoor activity; exposure to the index patient's blood and respiratory secretions or to grossly bloody oropharyngeal secretions; presence of skin lesions during exposure; whether skin surfaces were washed after exposure; whether they were exposed to the patient's stool or urine; and the timing of these events. Health care workers were asked about their use of masks and gloves.

Ethical and Human Subjects Review

The study was approved by the ethics committee of China CDC, according to the medical research regulations of Ministry of Health, China. Oral informed consent was obtained from all study participants.

Statistical Analysis

All statistical calculations were performed using Epi Info 6.04d (http://www.cdc.gov/epiinfo). To identify specific exposure risk factors, retrospective cohort comparisons were evaluated by calculating attack rates, relative risk, and 95% confidence intervals and by Fisher exact test; significance was defined as a 2-tailed $P<.05$.

RESULTS

Index Case

A 50-year-old woman with a 1-day abrupt onset of sudden fever (39.2°C), headache, myalgia, arthralgia, dizziness, and malaise presented to the village clinic on October 31, 2006, and was treated with ribavirin, cephalothin, dexamethasone, and amidopyrine for 4 days. At 9 PM on November 3, she was admitted to the local hospital because of gum bleeding, facial edema, nausea, vomiting, and oliguria, a temperature of 39.7°C, blood pressure of 85/60 mm Hg, and pulse rate of 96/min; a rash was noted over her trunk. Laboratory testing showed leukopenia (white blood cell count, 3300/μL), thrombocytopenia (platelet count, 18×10^3/μL), elevated serum aspartate aminotransferase

(629 U/L) and alanine aminotransferase (69 U/L), elevated creatinine (2.6 mg/dL), and elevated blood urea nitrogen (48 mg/dL) levels. Dipstick urinalysis revealed 3+ hematuria and 3+ proteinuria (protein, 3 g/L). (To convert aspartate aminotransferase and alanine aminotransferase to microkat per liter, multiply by 0.0167; creatinine to micromole per liter, by 88.4; and urea nitrogen to millimole per liter, by 0.357.)

Her condition progressively deteriorated, so she was transferred to a regional hospital at 11 AM, November 4. By 7 PM, the patient became obtunded, cyanotic, and purpuric and was bleeding from her nose and mouth. This extensive mouth and nose bleeding required frequent aspiration and contaminated the working area surfaces, health care workers, and family members who were with her. Family members assisted with patient care by wiping blood from the patient's mouth and nose, rinsing and reusing the same towels. By 7:38 PM the patient developed rapidly progressive dyspnea and worsening oxygen saturation and required endotracheal intubation. The patient remained hypoxic and hypotensive with multiorgan failure and copious bleeding from the nose and mouth. Despite all efforts, the patient died at 6:45 AM, November 5, 2006. The final diagnosis was hemorrhagic fever with renal syndrome, even though no IgG antibodies to Hantaan virus were detected. A postmortem examination was not performed, and no blood or tissue samples remained for retrospective laboratory testing.

Retrospective questioning of the patient's family revealed that she was bitten by a tick 12 days before onset of fever; she had killed several mice in her home 9 days before onset, and her husband had hunted and brought home "wild animal carcasses" 3 days before onset of illness. A timeline of events is shown in the FIGURE.

Nosocomial Cases of HGA

Between November 9 and 17, 2006, 9 patients were identified at the regional hospital with fever higher than 38.0°C (9 of 9 patients), myalgia (5 of 9), diarrhea (7 of 9), leukopenia (white blood cell count, 1200-3700/μL in 9 of 9), thrombocytopenia (platelets, 39-115 × 10^3/μL in all 9), and elevated serum aspartate aminotransferase and alanine aminotransferase (7 of 9) (TABLE 1). All patients had contact with the index patient, including 5 family members, 2 physicians, and 2 nurses who had accompanied or treated her between November 4 and 5 (Figure).

The initial secondary case experienced fever on November 9, 4 days after death of the index case, followed on November 11 by another patient, on November 12 by 3 patients, and on November 14 by 3 more patients. The last patient reported illness on November 17, 12 days after the death of the index patient. The patients were between 25 and 67 years (mean, 36.2 years), and 6 were men. All were previously healthy. The average incubation period was 7.8 days (range, 4-12 days). All had fever of at least 38.5°C for 1 to 6 days (mean, 4 days). Diarrhea was characterized as 1 to 3 loose stools per day persisting for 1 to 2 days. All patients had relative bradycardia. One patient developed acute respiratory distress syndrome as a complication of *Aspergillus* pneumonia and tuberculosis during his hospitalization. The other 8 patients were mildly affected, recovered, and were discharged in good health.

Contact Investigation

The index patient had contact with 63 persons after onset of illness: 21 family members and 42 health care workers. Of the 42 health care workers, 18 were from the local hospital, including 2 from the village clinic, and 24 were from the regional hospital. Of the 21 family members, 4 had contact with the index patient in only the local hospital, 13 only in the regional hospital, and 4 in both. The 9 secondary cases occurred among the 39 health care workers and relatives with patient exposure at the regional hospital, representing an attack rate of 23%. All 9 cared for the index case in the final 12 hours of her life while she was in the critical care unit and during the endotracheal intubation procedure. No one whose only contact with the index patient was before these 12 hours was infected.

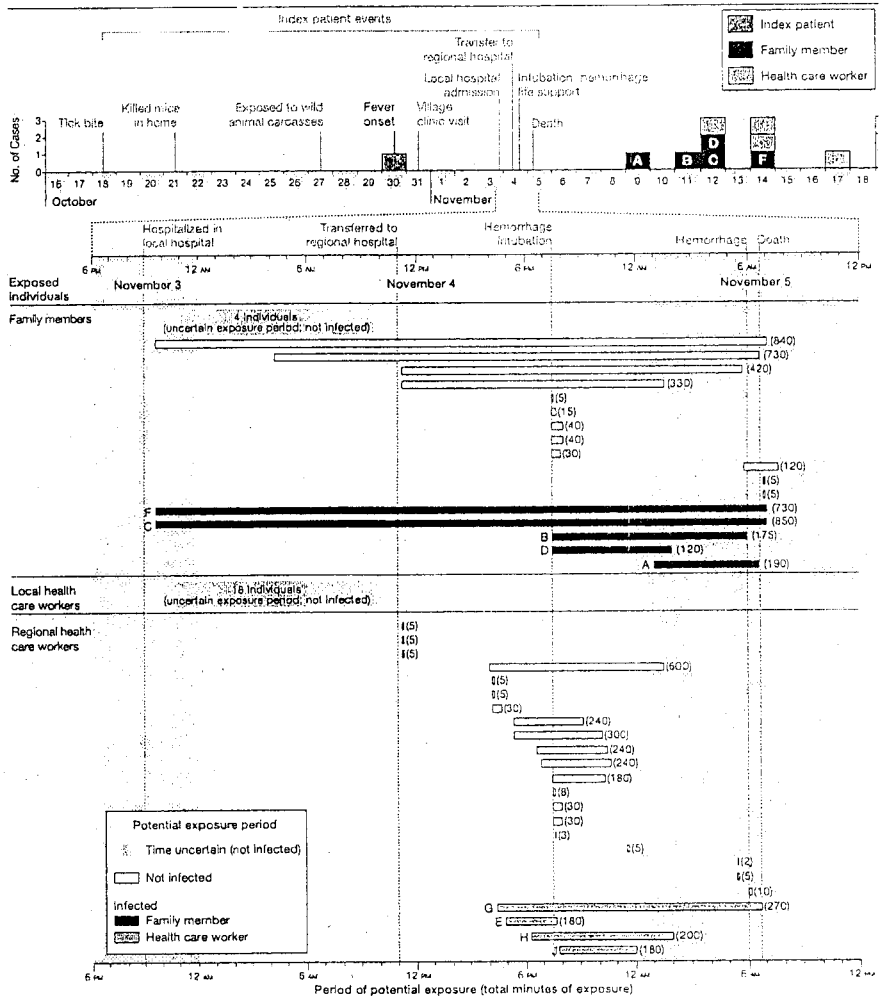
Serological and Molecular Diagnosis

Anaplasma phagocytophilum IgG seroconversions were detected for all 9 patients, and a 4-fold IgG titer increase was observed in 7 of 9 patients (Table 1). Nested PCR using genus-common *rrs* and species-specific *rrs* and *groEL* primers identified *A phagocytophilum* DNA in the blood samples from all 9 patients when they were in the acute phase, whereas all healthy and template controls had negative test results. The identity of amplicons from each of the 9 patients was confirmed by sequencing; all *rrs* sequences (206 base pairs) were identical and all *groEL* sequences (446 nt) were identical (GenBank accession numbers: *rrs* EF211110-17 and EF473210; *groEL* EF47320108 and EF473209). Although the *rrs* sequences were identical to most other human-derived strains globally, sequences from *groEL* were identical to some US strains (Wisconsin and New York) but differed from *A phagocytophilum* in China (93.6%; EU008083), Germany (99.4%; AY281850), and California (99.7%; U96727). These data support the premise that a single clone was responsible for all of the 9 secondary cases. Although peripheral blood smears were examined for all 9 patients at the time of illness, no convincing evidence of *A phagocytophilum* morulae was observed. All RT-PCR, PCR, antigen detection, and IgM antibody detection tests for microbial and viral etiologies were negative.

Risk Factors

The exposure data implicate transmission at the regional hospital, permitting focus on risk factors in 39 individuals, including 24 health care workers and 15 family members (TABLE 2). Two family members who

Figure. Timeline of Critical Events for the Index Patient and Direct Contact Intervals of Family Members and Health Care Workers With the Index Patient and Exposure of Patients With Nosocomial Human Granulytic Anaplasmosis



Top, epidemic curve showing progression of outbreak and key events during the index patient's illness. Bottom, each bar indicates the period of potential exposure while family members were in the hospital and while health care workers were assigned to care for the index patient. Duration of exposure in minutes is shown in parentheses and may not have occurred continuously during the exposure period. Capital letters designate the corresponding secondary cases in the top and bottom panels.

had contact with the index case after her death were not included.

None of the 9 secondary cases reported tick bites, exposure to wild animals, or participation in hunting activity in the preceding 2 months, and only 1 reported recent outdoor activity. For all 9 secondary cases, culture serological, antigen detection, and nucleic acid detection studies for other infectious etiologies were negative.

Of 24 regional hospital health care workers who had contact with the index patient, 18 were on duty during the final 12 hours, and 4 of the 18 who were involved in the endotracheal intubation were infected. Of these 4, 3 were involved in endotracheal intubation and care during times of hemorrhage. Sixteen of 24 health care workers (67%) from the regional hospital wore masks and 9 of 24 (38%) wore gloves.

Of 17 family members who reported contact with the index patient at the regional hospital, 13 were present during endotracheal intubation, 5 of whom were infected. Of these 5 individuals, 3 reported blood contamination of skin and possible mucocutaneous exposures, suggesting direct contact with blood or respiratory secretions as the mechanism of transmission.

Among the 28 individuals who reported close contact (≤50 cm) with the index patient during the final 12 hours of her life, 9 were infected. In contrast, none of the 11 individuals who reported a physical distance of more than 50 cm from the index patient during the same time was infected. The index patient was exposed to 20 contacts for more than 2 hours, and 9 were infected, whereas none of 19 contacts exposed fewer than 2 hours was infected. All 9 infected patients reported contact with blood ($P = .002$) and 7 had contact with respiratory secretions (relative risk, 7.0; 95% confidence interval, 1.7-29.1; Table 2). Those persons with skin expsoure to blood ($P < .001$) or respiratory secretions ($P = .004$), or those with preexisting skin lesions or injuries followed by exposure to blood (relative risk, 3.6; 95% confidence interval, 1.1-7.6; $P = .02$) were significantly more likely to be infected (TABLE 3). Neither exposure to stool nor exposure to urine from the index case resulted in increased risk (0.6 and 1.1, respectively).

Table 1. Clinical, Laboratory, and Serological Findings of 9 Patients With Nosocomial Human Granulocytic Anaplasmosis

	Infected Patients								
	2	3	4	5	6	7	8	9	10
Clinical findings[a]									
Days hospitalized	19	21	19	19	19	19	21	19	36
Temperature ≥38.5°C	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Malaise	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Chills	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Diarrhea	Yes	No	Yes	Yes	No	Yes	Yes	Yes	Yes
Myalgia	Yes	No	Yes	Yes	No	No	Yes	No	Yes
Coryza/pharyngitis	No	No	No	No	Yes	Yes	No	No	Yes
Headache	Yes	No	No	No	No	Yes	No	No	No
Nausea	No	No	Yes	No	No	No	No	No	Yes
Edema	No	No	No	No	No	Yes	No	No	No
Gum bleeding	No	No	No	No	No	Yes	No	No	No
Dysuria	No	No	No	No	No	No	No	No	Yes
Laboratory values									
Lowest blood count, range of normal									
White blood cell, 4500-11 000/μL[a]	2600	1900	2700	2100	2500	1200	1800	3700	2200
Platelet, 150-350 × 10^3/μL	46	49	85	39	115	47	40	52	42
Highest liver enzymes, U/L									
AST, men <38; women <32	252	116	ND	77	ND	50	50	77	78
ALT, men <40; women <31	84	66	ND	64	ND	89	89	74	139
Anaplasma phagocytophilum IgG titers									
Days after onset									
0-7	<64	<64	<64	64	64	<64	<64	<64	<64
20-25	ND	64	64	128	128	128	ND	64	128
55-70	256	256	<64	256	256	<64	64	128	ND
A Phagocytophilum PCR results									
rrs	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
groEL	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes

Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase; ND, not done; PCR, polymerase chain reaction.
[a] Clinical findings that were documented during the course of each patient's hospitalization.

COMMENT

Nine cases of *A phagocytophilum* infection were confirmed at the regional hospital in the Anhui Province of China in a 9-day period. All presented with HGA as described in North America and Europe[7] and fulfilled the US CDC laboratory criteria for the diagnosis of HGA.[15] The most remarkable aspect of these cases was that transmission was very unlikely to be tick-borne, but was closely associated with blood or respiratory secretion exposure from an index patient who died of a fulminant febrile illness with hemorrhage. Although the index patient can only be categorized as a possible case, clinical and historical support for the diagnosis of HGA is strong. She had a tick bite within the known incubation period and had a clinical presentation compatible with severe HGA.[4] Moreover, the epidemiological investigation of exposed individuals with HGA implicates her as the index case. Unfortunately, no tissue or serum sample is available to confirm retrospectively her diagnosis.

Human granulocytic anaplasmosis and human monocytic ehrlichiosis were initially identified with presentations now recognized as relatively uncommon for their natural histories.[16,19] Infection can be severe, with intensive care unit admission required in 7% of patients and fatalities occurring in up to 1%, yet most infections are sporadic and probably self-limited.[4] Based on the mild to moderate severity observed in 8 of the 9 secondarily infected patients, Chinese HGA conforms to the spectrum of clinical severity observed in North America.[4,7,15] The fatal outcome in the index case is clinically similar to that observed for other HGA fatalities, including exsanguination with sepsis syndrome possibly relating to cytokine overproduction, opportunistic infections, and increased HGA severity in the setting of preexisting medical conditions such as diabetes mellitus.[7,20]

A phagocytophilum transmission in China and Asia is predicated on the presence of this zoonotic agent in vector ticks and vertebrate hosts. Although studies in Asia are limited, at least 8 have examined *A phagocytophilum* infection of ticks, including 2284 *Ixodes persulcatus* ticks, of which 4.4% carried *A phagocytophilum* DNA, a prevalence similar to that in European and North American *Ixodes* species ticks.[12,14,21-27] Likewise, 9% and 24% of *Apodemus* species field mice in northern China and Korea, respectively, and 64% of *Crosidura lasiura* shrews in Korea are infected.[13,21,24,28,29] Although no proven cases of HGA have been previously identified in China, at least 1 study describes *A phagocytophilum* DNA in the blood of 4 Chinese patients with tick bites,[14,30] and seroepidemiological investigations demonstrate that 2% to 9% of febrile patients in Korea,[10,11] and between 0.5% and 6% of healthy Chinese residents have *A phagocytophilum* antibodies.[31]

Rare examples of nontick transmission of HGA exist in the literature and include direct exposure to deer blood,[32] transfusion,[33] and transplacental transmission.[34] Similarly, under the proper circumstances other rickettsial infections are transmissible via aerosol, direct contact with mucous

Table 2. Risk Factors for Acquisition of Human Granulocytic Anaplasmosis Among 39 Contacts Exposed to Index Patient While at the Regional Hospital

Exposure to Index Patient	No./Total (%)			
	Attack Rate With Exposure Factor	Attack Rate Without Exposure Factor	Relative Risk (95% Confidence Interval)[a]	*P* Value[b]
≤50 cm to nose and mouth	9/28 (32.1)	0/11 (0)		.04
>2 h	9/20 (45.0)	0/19 (0)		.001
During or after intubation	9/30 (30.0)	0/9 (0)		.09
During massive hemorrhage period	4/9 (44.4)	5/30 (16.7)	2.7 (0.9-7.9)	.17
Any direct blood contact	9/22 (40.9)	0/17 (0)		.002
Direct respiratory or tracheal secretion contact	7/13 (53.8)	2/26 (7.7)	7.0 (1.7-29.1)	.003

[a] Infinite or not able to be calculated.
[b] Fisher exact test (2-tailed).

Table 3. Risk Factors for Human Granulocytic Anaplasmosis Associated With Direct Exposure to Index Patient's Blood and Respiratory Secretions

Exposure Factor	No./Total (%)			
	Attack Rate With Exposure Factor	Attack Rate Without Exposure Factor	Relative Risk (95% Confidence Interval)[a]	*P* Value[b]
Any direct blood contact during hemorrhage				
On skin	9/12 (75.0)	0/10 (0)		<.001
Open wounds or abrasions	4/4 (100.0)	5/18 (27.8)	3.6 (1.1-7.6)	.02
Not washed timely	4/8 (50.0)	5/14 (35.7)	1.4 (0.5-3.8)	.66
Direct respiratory or tracheal secretion contact				
On skin	7/8 (87.5)	0/5 (0)		.004
Open wounds or abrasions	4/4 (100.0)	3/9 (33.3)	3.0 (1.2-7.6)	.07
Not washed timely	3/6 (50.0)	4/7 (57.1)	0.9 (0.3-2.4)	>.99

[a] Infinite or not able to be calculated.
[b] Fisher exact test (2-tailed).

membranes or conjunctivae, or mechanical fomite transmission.[35-38] Direct exposure to small blood volumes probably carries a low risk because experimental and natural infections of white-tailed deer result in only low-level bacteremia.[39] However, it is possible that this low risk may be offset by large volumes of animal blood and tissues, such as those to which butchers are exposed.

Another factor related to transmissibility is the blood burden of *A phagocytophilum*, which appears to increase with immunosuppression resulting in absolute infected neutrophil counts as high as $2.7 \text{ to } 5.9 \times 10^9$/L.[18,40] It is unclear to what degree the sustained dexamethasone treatment of the index case contributed to transmission. The final consideration is the likelihood of health care worker and family member exposure to sufficient volumes of infectious body fluids to account for transmission. It is not unusual for occupational blood exposure to occur among those caring for patients with hemorrhage or during procedures such as intubation or surgery, for which the relative risk is 3 to 4 times higher than for other medical specialties.[41] In western societies, most family members are excluded from these events and health care workers are increasingly protected by training and barriers such as gloves, gowns, and masks.[42] However, retrospective questioning of our cases clearly indicated that both family members and health care workers not only participated in these events but were unlikely to use gloves and so reported that body surfaces were contaminated by potentially infectious fluids. Moreover, many participants did not acknowledge use of postexposure precautions, such as hand and skin washing.

Although it is likely that routine blood and body fluid precautions will protect against such future events, strict adherence to protective protocols is mandatory even if communicability is deemed unlikely. The lessons of this study remain relevant to the daily hospital and health care unit operations to prevent any additional nosocomial outbreaks of HGA. Moreover, as China advances into its future, it must also now become prepared to deal with the increasing threat that tick-borne rickettsial pathogens have been already brought to the United States and Europe.

Author Contributions: Dr Xu had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis. Drs Zhang, Liu, Ni, Li, Y. Yu, and X. Yu contributed equally to this work.
Study concept and design: L. Zhang, Liu, Ni, Li, Y. Yu, Wan, Jing, Rui, Yang, Wang, Dumler, Feng, Ren, Xu.
Acquisition of data: Liu, Ni, D. Li, Y. Yu, Wan, Q. Li, Liang, Jiang, Jing, Rui, Luan, Fu, J. Zhang, Xu.
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Drafting of the manuscript: L. Zhang, Liu, Ni, Q. Li, Y. Yu, Wan, Liang, Jiang, Jing, Luan, Fu, J. Zhang, Dumler, Xu.
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Obtained funding: Rui, Wang, Xu.
Administrative, technical, or material support: Liu, Ni, D. Li, Y. Yu, X. Yu, Wan, D. Li, Liang, Jiang, Jing, Rui, Yang, Feng, Ren, Xu.
Study supervision: Wang, Dumler, Feng, Xu.
Financial Disclosures: Dr Dumler reports that he holds a patent for a method for in vitro propagation of *A phagocytophilum* for which royalty fees are paid. Otherwise no other authors report disclosures of financial or potential conflicts of interest.
Funding/Support: This work was supported by grants 2005CB522904 and 200802016 (Dr Xu) from the Ministry of Science and Technology and by emerging response found from National Institute of Communicable Disease Control and Prevention, China CDC, Beijing, China.
Role of the Sponsor: The sponsors provided funding, but had no role in determining study design, data collection, or interpretation.

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> Nothing is more estimable than a physician who, having studied nature from his youth, knows the properties of the human body, the diseases which assail it, the means which will benefit it, exercises his art with caution, and pays equal attention to the rich and the poor.
> —Voltaire (1694-1778)

HUMAN GRANULOCYTIC ANAPLASMOSIS IN CHINA

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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 2009年4月10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	第82回日本細菌学会総会 (2009年3月12日～14日)	公表国 日本	
販売名（企業名）	別紙のとおり					

研究報告の概要

問題点：retrospectiveな解析により、マダニ媒介性の新興感染症である「アナプラズマ症」のヒト感染症例が日本で初めて確認された。

「アナプラズマ症」は、1994年に米国で初めて確認されたマダニ媒介性の新興感染症で、その病原体はリケッチア目に分類される*Anaplasma phagocytophilum*である。本菌は、ヒトの顆粒球に特異的に感染して、発熱を伴ったリケッチア症様の疾患を引き起こす。我が国では、これまで「アナプラズマ症」のヒト感染症例は確認されていなかった。今回、*A. phagocytophilum*の感染が疑われる発熱性疾患患者を見出したので報告する。2002年～2003年に高知県で発生した発熱性疾患患者において、「日本紅斑熱」が疑われた18名の患者の血餅からDNAを抽出し、*A. phagocytophilum*に特異的な*p44/msp2*外膜蛋白遺伝子群を標的としたNested PCRを行った。その結果、2名の患者から*p44/msp2*遺伝子群のPCR産物が検出された。その後、得られた増幅産物をTAクローニングし、無差別にそれぞれ27個と40個の組換え体を選出して、塩基配列を決定し系統樹解析を行った。その結果、得られた*p44/msp2*クローンはそれぞれの患者に特異的なクラスターを形成することが判った。また、2名の患者のうちの1名は、「日本紅斑熱」起因細菌である*Rickettsia japonica*の16SrDNAもPCRにより増幅されたことから、この1名は、*A. phagocytophilum*と*R. japonica*の混合感染であることが判明した。以上、今回のretrospectiveな解析により、日本国内にも*A. phagocytophilum*感染による「アナプラズマ症」の存在が強く示唆された。よって、今後は、大規模な患者探索が望まれる。

報告企業の意見	今後の対応
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。

使用上の注意記載状況・その他参考事項等

記載なし

MedDRA/J ver.12.0

別紙

一般的名称	①人血清アルブミン、②人血清アルブミン、③人血清アルブミン*、④人免役グロブリン、⑤乾燥ペプシン処理人免疫グロブリン、⑥乾燥スルホ化人免疫グロブリン、⑦乾燥スルホ化人免疫グロブリン*、⑧乾燥濃縮人活性化プロテインC、⑨乾燥濃縮人血液凝固第Ⅷ因子、⑩乾燥濃縮人血液凝固第Ⅸ因子、⑪乾燥抗破傷風人免疫グロブリン、⑫抗HBs人免疫グロブリン、⑬トロンビン、⑭フィブリノゲン加第ⅩⅢ因子、⑮乾燥濃縮人アンチトロンビンⅢ、⑯ヒスタミン加人免疫グロブリン製剤、⑰人血清アルブミン*、⑱人血清アルブミン*、⑲乾燥ペプシン処理人免役グロブリン*、⑳乾燥人血液凝固第Ⅸ因子複合体*、㉑乾燥濃縮人アンチトロンビンⅢ
販売名（企業名）	①献血アルブミン20“化血研”、②献血アルブミン25“化血研”、③人血清アルブミン“化血研”*、④“化血研”ガンマーグロブリン、⑤献血静注グロブリン“化血研”、⑥献血ベニロンーⅠ、⑦ベニロン*、⑧注射用アナクトC2、500単位、⑨コンファクトF、⑩ノバクトM、⑪テタノセーラ、⑫ヘパトセーラ、⑬トロンビン“化血研”、⑭ボルヒール、⑮アンスロビンP、⑯ヒスタグロビン、⑰アルブミン20%化血研*、⑱アルブミン5%化血研*、⑲静注グロブリン*、⑳ノバクトF*、㉑アンスロビンP1500注射用
報告企業の意見	アナプラズマ症はマダニにより媒介される発熱性疾患で、その病原体は顆粒球に特異的に感染する0.2～2μmの大きさの球状もしくは楕円状の偏性寄生性のグラム陰性桿菌である。1994年、米国で発熱性疾患患者の好中球の中にエーリキア様細菌の感染が認められ、ヒト顆粒球エーリキア症病原体［Human Granulocytic Ehrlichiosis (HGE) agent］と呼ばれるようになった。その後、1996年にはその病原体が分離報告され、さらに2001年にはEhrlichia属からAnaplasma属へと配置換えされて、*Anaplasma phagocytophilum*という学名が付された。それに伴って、昨今ではその病名もヒト顆粒球アナプラズマ症［Human Granulocytic Anaplasmosis (HGA)］と呼ばれている。*A. phagocytophilum*は、ヒトの他、ウマやヒツジなどにも感染し、アナプラズマ症を引き起こすことから「人獣共通感染症」病原体としても知られている。(http://idsc.nih.go.jp/iasr/27/312/dj312d.html) *A. phagocytophilum*によるアナプラズマ症の発生は欧米が中心であるが、2006年に日本においても*A. phagocytophilum*がマダニから検出されたことが初めて報告された。 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、*A. phagocytophilum*よりも小さいウイルスの除去を目的とした平均孔径19nm以下の「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*A. phagocytophilum*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、これまでに本剤によるアナプラズマ症感染の報告例は無い。 以上の点から、本剤はアナプラズマ症感染に対して一定の安全性を確保していると考えるが、今後とも関連情報の収集に努め、本剤の安全性の確保を図っていきたい。

*現在製造を行っていない

P2-182　国内初の新興感染症「アナプラズマ症」について

○大橋 典男[1]，烏 日図[1]，高 娃[1]，川森 文彦[1,2]，高野 愛[3,4]，川端 寛樹[3,4]，安藤 秀二[5]，岸本 壽男[5]（静岡県大・食品栄養科学・微生物[1]，静岡県環衛研・微生物[2]，岐阜大学院・連合獣医[3]，国立感染研・細菌一[4]，国立感染研・ウー[5]）

「アナプラズマ症」は，1994 年に米国で初めて確認されたマダニ媒介性の新興感染症で，その病原体はリケッチア目に分類される *Anaplasma phagocytophilum* である。本菌は，ヒトの顆粒球に特異的に感染して，発熱を伴ったリケッチア症様の疾患を引き起こす。我が国では，これまで「アナプラズマ症」のヒト感染症例は確認されていなかった。今回，*A. phagocytophilum* の感染が疑われる発熱性疾患患者を見出したので報告する。2002 年～ 2003 年に高知県で発生した発熱性疾患患者において，「日本紅斑熱」が疑われた 18 名の患者の血餅から DNA を抽出し，*A. phagocytophilum* に特異的な *p44/msp2* 外膜蛋白遺伝子群を標的とした Nested PCR を行った。その結果，2 名の患者から *p44/msp2* 遺伝子群の PCR 産物が検出された。その後，得られた増幅産物を TA クローニングし，無差別にそれぞれ 27 個と 40 個の組換え体を選出して，塩基配列を決定し系統樹解析を行った。その結果，得られた *p44/msp2* クローンはそれぞれの患者に特異的なクラスターを形成することが判った。また，2 名の患者のうちの 1 名は，「日本紅斑熱」起因細菌である *Rickettsia japonica* の 16S rDNA も PCR により増幅されたことから，この 1 名は *A. phagocytophilum* と *R. japonica* の混合感染であることが判明した。以上，今回の retrospective な解析により，日本国内にも *A. phagocytophilum* 感染による「アナプラズマ症」の存在が強く示唆された。よって，今後は，大規模な患者探索が望まれる。

【非会員共同研究者：千屋誠造（高知衛研），福永和俊（高知衛研），船戸豊彦（室戸病院），浜宇津良治（中芸クリニック），塩尻正明（愛媛県立中央病院），中島秀樹（高知大）】

別紙様式第２－１

医薬品　研究報告　調査報告書

識別番号・報告回数			報告日	第一報入手日	新医薬品等の区分	総合機構処理欄
				2009 年 2 月 6 日	該当なし	
一般的名称	別紙のとおり	研究報告の公表状況	第 56 回日本ウイルス学会学術集会（2008 年 10 月 27 日）		公表国 日本	使用上の注意記載状況・その他参考事項等
販売名（企業名）	別紙のとおり					記載なし

研究報告の概要

問題点：日本国内の前立腺がん患者集団中に XMRV 感染の存在が示唆された。

XMRV (Xenotropic MuLV-related virus) は 2006 年に米国の前立腺がん患者で発見された新規 Gammaretrovirus である。感染している前立腺がん患者の 40%に RNaseL 遺伝子の一定の変異（QQ 変異）が報告されており、自然免疫の一端を担う RNaseL と XMRV 感染の関連が強く示唆されてきた。

日本国内の前立腺がん患者血清及び大阪府赤十字血液センターにおける感染症検査終了後の献血検体血清を用いて、ウェスタンブロット法で抗体の検出を、さらに、前立腺がん患者における抗体陽性血清について nested RT-PCR を行い XMRV 核酸検出を試みた。前立腺がん患者 30 名、献血者 120 名のスクリーニングを行ったところ、Gag に対する特異的抗体反応が前立腺がん患者 2 名、献血者 5 名の血清で認められた。Gag 抗体陽性の前立腺がん患者血清 1 検体よりウイルス核酸を検出した。

献血者及び前立腺がん患者の抗体反応性が HTLV で観察されたような HTLV-Gag-indeterminate pattern の類似現象である可能性を含め、更なる検討を続ける予定である。

報告企業の意見	今後の対応
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。

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別紙

一般的名称	①人血清アルブミン、②人血清アルブミン、③人血清アルブミン*、④人免役グロブリン、⑤乾燥ペプシン処理人免疫グロブリン、⑥乾燥スルホ化人免疫グロブリン、⑦乾燥スルホ化人免疫グロブリン*、⑧乾燥濃縮人活性化プロテインC、⑨乾燥濃縮人血液凝固第Ⅷ因子、⑩乾燥濃縮人血液凝固第Ⅸ因子、⑪乾燥抗破傷風人免疫グロブリン、⑫抗 HBs 人免疫グロブリン、⑬トロンビン、⑭フィブリノゲン加第ⅩⅢ因子、⑮乾燥濃縮人アンチトロンビンⅢ、⑯ヒスタミン加人免疫グロブリン製剤、⑰人血清アルブミン*、⑱人血清アルブミン*、⑲乾燥ペプシン処理人免役グロブリン*、⑳乾燥人血液凝固第Ⅸ因子複合体*、㉑乾燥濃縮人アンチトロンビンⅢ
販売名(企業名)	①献血アルブミン 20 “化血研”、②献血アルブミン 25 “化血研”、③人血清アルブミン “化血研” *、④ “化血研” ガンマーグロブリン、⑤献血静注グロブリン “化血研”、⑥献血ベニロンーⅠ、⑦ベニロン*、⑧注射用アナクトC2,500 単位、⑨コンファクトF、⑩ノバクトM、⑪テタノセーラ、⑫ヘパトセーラ、⑬トロンビン “化血研”、⑭ボルヒール、⑮アンスロビンP、⑯ヒスタグロビン、⑰アルブミン 20%化血研*、⑱アルブミン 5%化血研*、⑲静注グロブリン*、⑳ノバクトF*、㉑アンスロビンP 1500 注射用
報告企業の意見	XMLV が属するガンマレトロウイルス属はレトロウイルス科の 1 つで、多くの種ががん遺伝子を有し、肉腫や白血病を引き起こす。ガンマレトロウイルス属の代表的ウイルスには、マウス白血病ウイルス（MuLV）がある。ガンマレトロウイルス属ウイルスは、一本のプラス鎖 RNA を核酸として持ち、直径 80～100nm でエンベロープを有している。 本剤の製造工程には、冷エタノール分画工程、ウイルス除去膜ろ過工程あるいは加熱工程等の原理の異なるウイルス除去及び不活化工程が存在しているので、ウイルスクリアランスが期待される。各製造工程のウイルス除去・不活化効果は、「血漿分画製剤のウイルスに対する安全性確保に関するガイドライン（医薬発第 1047 号、平成 11 年 8 月 30 日）」に従い、ウシウイルス性下痢ウイルス（BVDV）、仮性狂犬病ウイルス（PRV）、ブタパルボウイルス（PPV）、A 型肝炎ウイルス（HAV）または脳心筋炎ウイルス（EMCV）をモデルウイルスとして、ウイルスプロセスバリデーションを実施し、評価を行っている。今回報告した XMLV は、エンベロープの有無、核酸の種類等からモデルウイルスとしては BVDV が該当すると考えられるが、上記バリデーションの結果から、本剤の製造工程がこれらのウイルスの除去・不活化効果を有することを確認している。また、これまでに本剤による XMLV の感染の報告例は無い。 以上の点から、本剤は XMLV 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

献血者ならびに前立腺がん患者における新規ヒトレトロウイルス XMRV に対する血清学的解析

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【目的と意義】
XMRV は 2006 年に米国の前立腺がん患者で発見された新規 Gammaretrovirus である。感染している前立腺がん患者の 40%に RNaseL 遺伝子の一定の変異（QQ 変異）が報告されており、自然免疫の一端を担う RNaseL と XMRV 感染の関連が強く示唆されてきた。本研究では、日本の前立腺がん患者および献血者における XMRV 感染の有無を把握し、血液事業に対する影響を評価するとともに、前立腺がんと XMRV 感染の関連を追跡することを目的とする。

【材料と方法】
XMRV プラスミドクローン VP62 を 293T 細胞にトランスフェクションし、培養上清中に放出されたウイルスを不活化したのち、スクリーニング用抗原とした。ELISA 法でのバックグラウンドが高かったため、スクリーニングはウエスタンブロット法で行った。被検体は（1）インフォームドコンセントを得た前立腺がん患者血清、および（2）大阪府赤十字血液センターにおける感染症検査終了後の献血検体血清を用いた。さらに、前立腺がん患者における抗体陽性血清について nested RT-PCR を行い XMRV 核酸検出を試みた。

【結果】
これまでに、前立腺がん患者 30 名、献血者 120 名のスクリーニングを行ったところ、Gag に対する特異的抗体反応が前立腺がん患者 2 名、献血者 5 名の血清で認められた。Gag 抗体陽性の前立腺がん患者血清 1 検体よりウイルス核酸を検出した。

【考察】
日本国内の前立腺がん患者集団中に XMRV ウイルス感染の存在が示唆された。用いたウイルス抗原の Env に対する反応性が見られなかった原因として Env 量が極めて少ない、もしくは用いたクローンの Env とは交差反応しない可能性があり、現在これらの判定を進めている。献血者および前立腺がん患者の抗体反応性が HTLV で観察されたような HTLV-Gag-indeterminate pattern の類似現象である可能性を含め、更なる検討を続け予定である。

別紙様式第2-1

No. 2

医薬品 研究報告 調査報告書

識別番号・報告回数			報告日	第一報入手日	新医薬品等の区分	総合機構処理欄
				2008. 11. 20	該当なし	
一般的名称	新鮮凍結人血漿		研究報告の公表状況	Lessa F, Leparc GF, Benson K, Sanderson R, Van Beneden CA, Shewmaker PL, Jensen B, Arduino MJ, Kuehnert MJ. Transfusion. 2008 Oct;48(10):2177-83.	公表国 米国	
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)					

研究報告の概要

○ルーチンの細菌培養スクリーニングの実施にもかかわらず、細菌に汚染されたプール血小板の輸血が原因となったC群連鎖球菌感染死亡症例

背景:慢性骨髄単球性白血病の高齢男性が、全血8本から製造したプール血小板(PLT)の輸血後48時間以内に呼吸困難を発現し死亡した。当該受血者の血液及びバッグに残存したプールPLTの培養でC群連鎖球菌(GCS)が生育したため、感染源と検査が偽陰性となった原因を調査した。

試験デザインおよび方法:関連した8本の赤血球(RBC)の培養を行い、また、関連供血者の検体を入手した。16SのrRNAとパルスフィールドゲル電気泳動(PFGE)により分離株を特定した。血液センターのスクリーニング方法についても調査した。

結果:死亡した男性とRBC8本のうち1本から培養されたベータ溶血性GCSが一致した。供血から20日後に採取した当該供血者の咽頭スワブはGCS陽性であり、*Streptococcus dysgalactiae* subsp. *equisimilis*と同定された。受血者、RBC、残存PLTと供血者咽頭スワブの分離菌はPFGEで区別できなかった。供血者は供血前後の症状や感染について否定した。血液センターのPLT細菌スクリーニングは、検出限界が1バッグ当たり15 CFUの市販の細菌検出システム(BacT/ALERT, bioMérieux)を使用して行われていた。

結論:PLTのGCS汚染原因として、無症候の供血者の関与が示唆された。現在の検査法は、すべての細菌汚染を検出するのに十分ではなく、特に培養量が制限されるプールPLTでは難しい。PLTの細菌汚染検出の向上が求められる。

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」

血液を介するウイルス、細菌、原虫等の感染
vCJD等の伝播のリスク

報告企業の意見	今後の対応
ルーチンの細菌培養スクリーニングを実施したプール血小板の輸血を受けた患者が、呼吸困難を発症、死亡し、患者血液、製剤及び無症候の供血者からC群連鎖球菌が検出されたとの報告である。	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、輸血情報リーフ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。今後も細菌やウイルスの検出や不活化する方策について情報の収集に努める。

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JRC2008T-070

TRANSFUSION COMPLICATION

Fatal group C streptococcal infection due to transfusion of a bacterially contaminated pooled platelet unit despite routine bacterial culture screening

Fernanda Lessa, German F Leparc, Kaaron Benson, Roger Sanderson, Chris A. Van Beneden, Patricia L. Shewmaker, Bette Jensen, Matthew J. Arduino, and Matthew J. Kuehnert

BACKGROUND: An elderly man with chronic myelomonocytic leukemia developed respiratory distress and died less than 48 hours after transfusion of a pool of eight whole blood–derived platelets (PLTs). Blood cultures from the recipient and cultures of remnants from the pooled PLT bag grew group C streptococci (GCS). An investigation was conducted to identify both the infection's source and the reasons for the false-negative screening result.

STUDY DESIGN AND METHODS: Red blood cell (RBC) units (cocomponent from the eight donations) were traced, quarantined, and cultured. Specimens from the implicated donor were obtained. Isolates were identified and typed by 16S rRNA and pulsed-field gel electrophoresis (PFGE). The blood center screening method was reviewed.

RESULTS: β-Hemolytic GCS, cultured from 1 of 8 RBC units, linked the fatal case to a single donor. The donor's throat swab collected 20 days after donation was positive for the presence of GCS, identified as *Streptococcus dysgalactiae* subsp. *equisimilis*. Isolates from the recipient, RBC unit, residual PLTs, and donor's throat swab were indistinguishable by PFGE. The donor denied any symptoms of infection before or after donation. PLT bacterial screening at the blood center was performed using a commercially available bacterial detection system (BacT/ALERT, bioMérieux) with a threshold of 15 colony-forming units per bag.

CONCLUSION: An asymptomatic donor was implicated as the source of GCS-contaminated PLTs. Current screening methods for PLTs are not sufficient to detect all bacterial contamination. Pooled PLTs are a particular challenge because the small volume of individual units places limits on culturing strategies. Improved detection of bacterial contamination of PLTs is needed.

Bacterial infection due to transfusion of contaminated platelet (PLT) components is an important patient safety concern.[1,2] Before the adoption of a standard requiring blood collection and transfusion service members to limit and detect bacterial contamination in all PLT components by AABB in 2004,[3] the estimated rate of bacterial contamination of PLT products ranged from 1 in 2000 to 1 in 3000 PLT units,[4] although the frequency of recognized sepsis from these products is much lower. Not all bacterially contaminated PLT units will result in a clinically recognized septic reaction; thus, the estimated rate of transfusion-related sepsis (1 in 100,000 units) for pooled PLTs before 2004 is likely to represent a substantial underestimation of the

ABBREVIATIONS: GCS = group C streptococci; PFGE = pulsed-field gel electrophoresis; WB = whole blood.

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This investigation was supported by the Office of Workforce and Career Development, Centers for Disease Control and Prevention.

Disclaimer: The findings and conclusions in this report are those of the authors and do not necessarily represent the views of the Department of Health and Human Services.

Received for publication February 26, 2008; revision received March 31, 2008, and accepted April 2, 2008.
doi: 10.1111/j.1537-2995.2008.01802.x
TRANSFUSION 2008;48:2177-2183.

problem.[5] Implementation of routine bacterial screening by blood centers represents an important advance toward ensuring the safety of PLT components. It does not, however, eliminate the risk of transfusion-related sepsis and death.[2,6,7] Current bacterial screening methods for PLTs have different levels of sensitivity, and none of them is likely to detect all pathogens.[8] Although culture is considered one of the best bacterial screening methods available, false-negative culture results can occur that lead to transfusion of bacterially contaminated blood components.[7]

Bacteria that contaminate blood products may originate from donor skin flora, from donor asymptomatic bacteremia, or from contamination during blood processing.[9-11] Most pathogens reported as causes of transfusion-related sepsis are organisms associated with skin contaminants,[2,5,7] suggesting that contamination is more likely to occur at the time of collection.

In this article, we report a PLT unit with a false-negative bacterial detection screening result. The event resulted in the death of the recipient by an unusual organism not previously associated with transfusion-related sepsis. An investigation was conducted to determine both the source of PLT contamination and the reasons for the false-negative screening result.

CASE REPORT

In April 2007, public health officials at the Florida Department of Health were notified of a fatal group C streptococcal infection after blood transfusion. The Centers for Disease Control and Prevention (CDC) was invited to assist in the investigation and the Food and Drug Administration (FDA) was notified of the potential transfusion-associated fatality. The patient was a 67-year-old man with refractory leukemia who received a pool of eight whole blood (WB)-derived PLTs. The patient was diagnosed with chronic myelomonocytic leukemia in April 2006. He never responded to chemotherapy treatments and required frequent transfusions. On April 16, 2007, when the patient presented to an outpatient infusion center to receive a PLT transfusion, his PLT count was 5×10^9 per L and he had no symptoms of infection. He received a pool of 8 (instead of the usual 8) WB-derived PLT units because of his previous history of poor response to PLT transfusions. No medication was given before transfusion. The PLT units were screened for bacteria using blood culture media (BacT/ALERT bottle, bioMérieux, Durham, NC), and no growth was observed after 5 days of incubation in the instrument.

At the end of the transfusion, the patient had chills for which a narcotic analgesic was administered. One hour after the transfusion was completed the patient became tachycardic, hypotensive, and hypoxic. The patient was transferred to the intensive care unit where his clinical status rapidly deteriorated, requiring ventilatory support and vasopressor agents. Sepsis was suspected, and broad-spectrum antibiotics were begun after blood cultures were collected. The following day, the patient's condition continued to worsen. He died less than 48 hours after the transfusion.

The patient's blood cultures were positive 1 day after the collection and showed Gram-positive cocci in pairs and in short chains, later identified as group C streptococci (GCS). Because the patient had onset of his illness soon after receiving PLTs, transfusion-related bacterial infection was suspected and an investigation was initiated.

MATERIAL AND METHODS

Culturing of blood components

The patient received PLTs derived from units of WB from eight different donors pooled by the hospital just before the time of transfusion. Seven of the 8 WB-derived PLT units were 3 days old, and 1 was 4 days old at the time of transfusion. Cultures of remnants from the pooled bag and of residual PLTs from each of the eight 50-mL individual-donor PLT bags were obtained. Cultures were performed at the hospital microbiology laboratory using the bacterial detection system (BacT/ALERT) for the recipient's blood and both chocolate agar plate and non-automated broth culture for residual PLTs and remnants of the pooled bag.

Cocomponents from each of the eight donations, including red blood cells (RBCs) and fresh-frozen plasma, were traced and quarantined. An 8-mL sample from each of the 8 RBC units was obtained and cultured by the blood center in the bacterial detection system.

Donor investigation and culturing

The implicated donor was interviewed, and specimens for culturing were collected including blood and swabs from throat, nose, antecubital skin, and perineal areas.

Isolate characterization

Isolates were submitted to the CDC for identification and typing. The isolates were characterized phenotypically using a conventional biochemical identification scheme and a rapid identification system (Rapid ID 32 Strep system, bioMérieux).[12-14] Comparative 16S rRNA gene sequencing[15] and pulsed-field gel electrophoresis (PFGE) analysis were performed as previously described.[16,17] PFGE patterns were analyzed with computer software (Bionumerics, Applied Maths, Inc., Austin, TX). A dendrogram was generated using unweighted pair group with arithmetic means and the Dice coefficient with a position tolerance of 1.25 percent and an optimization of 0.5 percent.

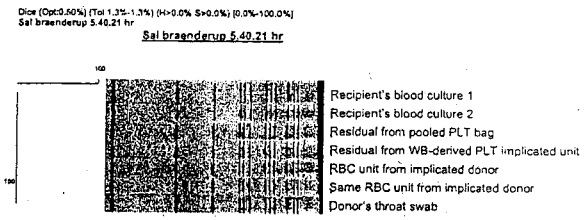

Fig. 1. PFGE and dendrogram of *S. dysgalactiae* subsp. *equisimilis* isolates recovered from the recipient's blood, a pooled PLT bag, an individual PLT unit, and the donor's RBC unit and throat swab, Florida, 2007.

Blood center screening method and validation test

Because the WB-derived PLT units used to make the pool were screened for bacterial contamination before being released, the screening method and the quality control (QC) test validation were reviewed.

RESULTS

Culturing of blood components

Cultures of the remnants from the pooled bag and of the residual PLTs from four of the eight individual PLT bags grew Gram-positive cocci later identified as GCS. The remaining RBC units, cocomponents of the eight WB-derived PLTs, were still available at the blood center and one of these RBC units also grew GCS. The presence of GCS in one RBC cocomponent, in addition to the WB-derived PTL units, allowed the event to be linked to a single donor.

Donor investigation and culturing

The implicated blood donor was a healthy 18-year-old girl with no history of illness in the 2 weeks before or since donation. She denied exposure to any sick people before donation and reported living with her parents, both of whom were apparently healthy. She had a history of four prior WB donations in the previous 19 months. In two of these four prior donations, WB-derived PLT was prepared, cultured negative, and transfused uneventfully. Culture of the donor's throat swab taken approximately 20 days after donation was positive for the presence of GCS. All other cultures from the donor failed to demonstrate GCS growth.

CDC laboratory results

The β-hemolytic *Streptococcus* specimen isolated from recipient's blood, remnants from the pooled bag, RBC unit, and donor's throat swab were confirmed to possess the Lancefield group C antigen. The conventional biochemical test reactions and the rapid identification system results were identical for all isolates, and the isolates were identified as *Streptococcus dysgalactiae* subsp. *equisimilis*. The 16S rRNA sequences were identical for all strains. Comparative 16S rRNA sequence analysis with reference strain 16S sequences in the CDC *Streptococcus* database showed the highest similarity (99.86%) to *S. dysgalactiae* subsp. *equisimilis*. PFGE analysis revealed that all isolates were indistinguishable (Fig. 1).

Review of blood center screening method and validation test

The following methods describe the procedure for blood donation preparation and PLT culture screening performed at the blood center. Before blood collection, the antecubital area is scrubbed for 30 seconds using a single-use applicator with a solution of 2 percent (wt/vol) chlorhexidine gluconate and 70 percent (vol/vol) isopropyl alcohol (ChloraPrep, Enturia, Inc., Leawood, KS). Blood collection is then performed using a single-use blood collection kit (Fenwal, Chicago, IL).

After the separation of PLTs from PLT-rich plasma, units are rested at room temperature (e.g., 20-24°C) for 2 hours. The units have an integrally attached tubing segment 9 to 12 inches in length. After the resting period is completed, the attached tubing segment containing between 1.6 and 2.4 mL of PLT-rich plasma is stripped and refilled three times to ensure that the tubing is filled with PLT-rich plasma that is representative of the content of the bag. The segments are then sealed and labeled with the corresponding unit number, cut, and placed in an incubator at 37°C for 24 hours. This subsequent incubation is performed to accelerate the bacterial growth in the segments as demonstrated previously.[18] At the completion of the incubation time, the segments are welded to a sampling harness using a sterile connecting device (TSCD, Terumo Medical Corp., Sommerset, NJ). The content of up to six segments is drawn from the segments using the syringe in the harness (Fig. 2). The syringe content is then inoculated into a single aerobic blood culture bottle (BacT/ALERT), and the bottle is incubated for 5 days for bacterial growth. PLT units are released if no growth is detected after 12 hours of incubation in the culture bottle. A final interpretation on the culture bottle is made after 5 days of incubation at 37°C.

The test for the detection of bacterial contamination was validated by spiking studies using pellets with standardized concentration (EZ-CFU, MicroBiologics, St Cloud, MN) of *Staphylococcus epidermidis* (ATCC 12228), *Escherichia coli* (ATCC 8739), and *Staphylococcus*

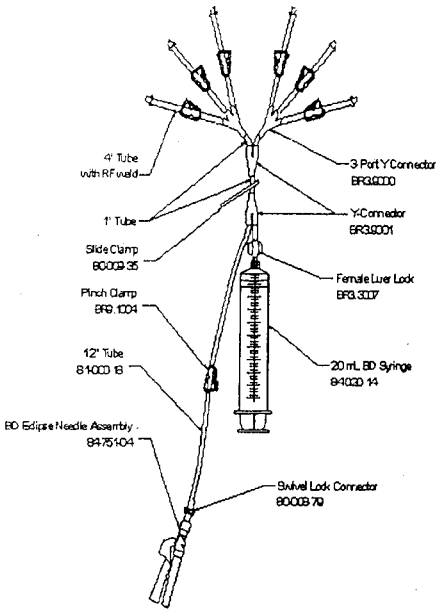

Fig. 2. Procedure for pooled PLT culture screening performed at the blood center. The syringe in the harness is used to draw the contents of up to six tubing segments containing PLT-rich plasma.

aureus (ATCC 6538).[19] During these validation tests, the detection limit for bacterial contamination was shown to be approximately 15 colony-forming units (CFUs) per bag.

DISCUSSION

This is the first reported case of infection and death due to transfusion of GCS-contaminated PLTs. β-Hemolytic GCS are pathogenic to humans and other mammals.[20-23] Although lesser known than groups A and B streptococci, both group C and group G streptococci are part of skin, oral cavity, nasopharynx, gastrointestinal, and vaginal normal flora.[24,25] Invasive infections due to GCS have been increasingly recognized,[21,22] likely due to improvement in diagnostic laboratory techniques and improved reporting. The most common species of GCS isolated in human infections is *S. dysgalactiae* subsp. *equisimilis*.[22,23,26] Outbreaks of pharyngitis by GCS have been reported, especially among college students;[23,27] invasive infection by these microorganisms in otherwise healthy people is less common and includes skin and soft tissue infections (e.g., cellulitis, erysipelas), septic arthritis, abscesses, osteomyelitis, infective endocarditis, and bacteremia. Population-based surveillance for streptococcal infections in Denmark and Canada have shown that the incidence of invasive GCS infection ranges from 0.4 to 0.5 per 100,000 inhabitants per year, with a higher prevalence in persons older than 60 years of age or with underlying conditions.[28,29]

This fatal transfusion reaction associated with a false-negative screening test highlights the residual risk of sepsis and death from PLT units screened for bacterial contamination. Several factors could explain the reason for the negative culture result after 5 days of incubation in the blood culture bottle (BacT/ALERT): 1) the sampling process may have been inadequate and too little volume from individual WB-derived PLT bags was available in the tubing segments, resulting in no viable organisms in the culture bottle; 2) insufficient volume from the syringe may have been inoculated into a single aerobic culture bottle;[7,30] or 3) the bacterial load of PLT unit at the time of testing was below the detection limit of the blood center screening process (i.e., 15 CFUs/bag).

Although GCS also has been reported as a skin contaminant,[25,28] introduction through phlebotomy is less likely due to the aseptic processes used for venipuncture. Contamination of the PLT unit was probably due to bacteremia in the donor, although she had no clinical manifestations of skin or pharyngeal disease when evaluated. The implicated donor, an apparently healthy young girl, likely developed transient asymptomatic bacteremia due to the presence of GCS in her oral cavity at the time of donation. As a result of the investigation, this donor has been indefinitely deferred for blood donation.

GCS was also isolated from three other PLT units besides the implicated donor's unit. A probable explanation for this is that if the fifth PLT unit pooled in the bag by the hospital was from the implicated donor, this unit may have contaminated the port of the pooling bag, subsequently contaminating PLT Units 6, 7, and 8.

Persistence of bacterial growth in contaminated PLT components occurs due to the relatively warm storage temperature of PLT units. At 20 to 24°C, a small bacterial inoculum can grow quickly, resulting in a large number of organisms in the PLT unit by the time of transfusion. Because this rapid bacterial growth occurs under normal PLT storage conditions, older units (≥5-day storage) are more likely to have higher bacterial load than younger units (≤5-day storage). Because of this phenomenon, FDA mandates that the storage period of WB-derived PLT units cannot be longer than 5 days. More septic reactions including fatalities have been reported with older PLT units.[7] Interestingly, the fatal GCS case reported in this article was caused by a PLT unit transfused on Day 3 after collection, suggesting a very rapid bacterial growth during storage and hence a high bacterial load in this recently collected unit; this phenomenon has previously been noted in association with Gram-negative organisms.[3]

Detection of bacterial contamination in pooled WB-derived PLTs remains a challenge. Because of the short storage time for WB-derived PLTs (i.e., 5 days), the blood center in our investigation performs sampling within 2 hours after separation of the components. This technique does not allow for an additional 24-hour holding period to improve the sensitivity of the test. Both the shorter holding period and the smaller sampling size (i.e., 1.6 to 2.4 mL in each tubing segment) are likely to decrease the sensitivity of the method when compared to comparable apheresis testing procedures. The sensitivity of the method described, however, is likely to be superior to pH and glucose measurements commonly used for WB-derived PLTs QC. At the blood center reporting this case, the overall incidence of true-positive bacterial contamination (i.e., confirmed by replicate growth on the units from which the tubing segment was obtained) using the method described is 1 in 21,000 WB-derived PLT units[18] (which can be estimated as 1 in 3500 WB-derived PLT pools if we assume that 1 segment in each pool of 6 was contaminated), whereas the incidence of true-positive bacterial detection on apheresis PLT units at this same institution is 1 in 2700.

Although alternative devices for prepooling and sampling for culture have been approved by the FDA,[31] these alternatives, as currently configured, require the use of proprietary blood collection bags, leukoreduction filter, and bacterial growth detection systems that are not compatible with the bacterial detection systems used at all blood establishments, including the blood establishment where the PLTs in this report were prepared.

The BacT/ALERT culture method was approved by the FDA in 2002 for QC of bacterial contamination of single-donor PLT (SDP) units only. Because use of the BacT/ALERT method for individual WB-derived PLT units is not practical due to the small volume of each unit, a study was conducted in 2005 to validate the use of this method for the detection of bacterial contamination in WB-derived PLTs in a pooled format.[32] This study demonstrated that the BacT/ALERT method is capable of detecting very low concentrations of bacteria in a single WB-derived PLT unit when the contaminated unit is pooled with 5 other sterile units for culturing. In this validation study, both aerobic and anaerobic bottles were used. Although the use of one aerobic bottle and one anaerobic bottle is strongly recommended by the manufacturers of BacT/ALERT, the majority of the blood centers only use one aerobic bottle[33] as reported in our investigation. A recent study done by Brecher and Hay[34] using *Staphylococcus lugdunensis* suggested that the use of both aerobic and anaerobic bottles may significantly increase sensitivity of screening, particularly when the inoculum is low. It is unclear, however, whether this increase in sensitivity is due to the use of anaerobic media or simply reflects an increase in total volume inoculated.

Non-culture-based screening methods have been suggested for detection of bacterial contamination in WB-derived PLT units;[8] however, these methods are typically less sensitive than culture. FDA recently approved a rapid test to be used to supplement current screening strategies for detection of bacterial contamination in PLTs.[35] This supplemental test is to be used near the time of transfusion and can detect bacterial contamination that was not detected by culture. The performance of this new test in WB-derived PLTs is unknown, however, since studies were conducted using leukoreduced apheresis PLTs.

Our report and others[2,6,7,36] indicate that current screening methods to prevent transfusion of bacterially contaminated PLTs can be improved. Further studies to evaluate the sensitivity of culture and non–culture-based screening methods for detection of bacterial contamination in WB-derived PLTs are needed. Efforts to improve recognition of bacterial contamination of PLTs also need to continue. If transfusion-related bacteremia is suspected, the residual blood product unit should be saved by the hospital and the blood center immediately informed. Timely information will allow blood centers to rapidly trace and quarantine potentially contaminated cocomponents made from the same donation. Finally, the BacT/ALERT package insert's recommendations should be followed, particularly concerning the use of one aerobic and one anaerobic culture bottle with sufficient volume. β-Hemolytic streptococci are facultative anaerobes and may be better recovered under anaerobic conditions.[37]

ACKNOWLEDGMENTS

The authors acknowledge Bernard Beall, PhD, Roberta Carey, PhD, Roger Morey, and Arnie Steigerwalt of the Centers for Disease Control and Prevention and Maria Calcaterra, BS, MT, of the Florida Department of Health, for their laboratory support.

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別紙様式第2-1

No. 11

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. 2. 18	該当なし	
一般的名称	乾燥濃縮人血液凝固第Ⅷ因子		研究報告の公表状況	Health Protection Agency, 2009 Feb 17. Available from: http://www.hpa.org.uk/webw/HPAweb&HPAwebStandard/HPAweb_C/1234859690542?p=1231252394302	公表国 英国	
販売名(企業名)	クロスエイトM250(日本赤十字社) クロスエイトM500(日本赤十字社) クロスエイトM1000(日本赤十字社)					

研究報告の概要

○血友病患者の剖検時にvCJD異常プリオンタンパク質が発見された
vCJDとは関係のない疾患により死亡した血友病患者(年齢70歳以上)の剖検時に、患者の脾臓からvCJDの異常プリオンタンパク質感染の証拠が見つかった。この患者は、生前vCJD及び神経学的症状は示していなかった。
英国健康保護局は、英国血友病センター医師会と共同し、現在詳細調査中であるこの予備情報が出血性疾患患者すべてに確実に伝わるよう尽力しているが、この新たな知見により血友病患者の看護や治療の方法が変わることはない。
伝播経路の調査は継続中であり最終的な見解はまだ得られていない。
当該患者は、vCJDに関する血液安全性改善措置が導入された1999年以前に、英国内で供血された凝固因子製剤による治療を受けたことが判明しており、その中に供血の6ヶ月後にvCJDの症状を発現した供血者由来血漿から製造された第VIII因子製剤1バッチが含まれていた。
血友病患者または血漿分画製剤の治療を受けた患者にvCJD異常プリオンタンパク質が見つかったのはこれが初めてである。
血友病患者は、すでに「公衆衛生上vCJDリスクを有する状態」に分類されることが医師から知らされているが、リスクの状態が変更されるものではない。
この新たな知見は、これまで理論上のリスクであったものが、血漿分画製剤を投与された特定の個人に対する現実のリスクとなる可能性を示すものと考えられるが、当該リスクはまだ非常に低いであろうと考えられる。
1999年以降、凝固因子製剤製造に英国内の血漿は使用されておらず、必要な患者には遺伝子組換製剤が使用されている。

使用上の注意記載状況・その他参考事項等

クロスエイトM250
クロスエイトM500
クロスエイトM1000

血液を原料とすることに由来する感染症伝播等
vCJD等の伝播のリスク

海外症例報告:
2009年03月05日付3-08000044

報告企業の意見	今後の対応
英国でvCJDとは関係のない疾患により死亡した血友病患者の剖検時に、初めてvCJDの異常プリオンタンパク質感染の証拠が見つかり、当局はすべての出血性疾患患者への情報提供と伝播経路の調査を実施しているとの報告である。	プリオン病の原因とされる異常プリオンが分画製剤製造工程で効果的に除去されるとの成績と併せて、これまでの疫学研究では如何なるプリオン病も、血漿分画製剤を介して伝播するという証拠は無かった。しかし、原因が特定されていないものの、本報告で初めて、第Ⅷ因子製剤を介してvCJDに感染する可能性が示唆された。引き続きプリオン病に関する新たな知見及び情報を収集するとともに、血漿分画製剤の製造工程における病原因子の除去・不活化技術の向上に努める。 なお、日本赤十字社は、CJD、vCJDの血液を介する感染防止の目的から、献血時に過去の海外渡航歴(旅行及び居住)、CJDの既往歴(本人、血縁者)、hGH製剤投与の有無を確認し、該当するドナーを無期限に献血延期としている。

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MedDRA/J Ver.11.0.1

Health Protection Agency

Protecting people
Preventing harm
Preparing for threats

JRC2009T-007

vCJD abnormal prion protein found in a patient with haemophilia at post mortem

17 February 2009

Evidence of infection with the agent (abnormal prion protein) that causes variant Creutzfeldt-Jakob Disease (vCJD) has been found at post mortem in the spleen of a person with haemophilia.

The patient, who was over 70 years old, died of a condition unrelated to vCJD and had shown no symptoms of vCJD or any other neurological condition prior to his death. The vCJD abnormal prion protein was only identified during post mortem research tests.

The Health Protection Agency is working with the UK Haemophilia Centre Doctors Organisation to ensure all patients with bleeding disorders are made aware of this preliminary information which is being further investigated. This new finding will not change the way patients with haemophilia are cared for or treated.

A final view as to how vCJD abnormal prion protein was transmitted to this haemophilia patient has yet to be reached because investigations are continuing to determine the most likely route of transmission. It is known that the patient had been treated with several batches of UK sourced clotting factors before 1999, which is when measures to improve the safety of blood in relation to vCJD were introduced. The patient's treatment had included one batch of Factor VIII that was manufactured using plasma from a donor who went on to develop symptoms of vCJD six months after donating the plasma in 1996.

This is the first time that vCJD abnormal prion protein has been found in a patient with haemophilia, or any patient treated with plasma products. This new finding, however, does not change the public health vCJD 'at risk' status of patients with bleeding disorders.

Haemophilia patients have previously been informed by their doctors of their possible increased risk of exposure to vCJD via clotting factors. In 2004 all patients with bleeding disorders who had been treated with UK-sourced pooled plasma products between 1980 and 2001 were told that, owing to potential vCJD infectivity from these products they were to be classified as at-risk of vCJD for public health purposes.

Professor Mike Catchpole, Director of the Health Protection Agency's Centre for Infections, said:

"This new finding may indicate that what was until now a theoretical risk may be an actual risk to certain individuals who have received blood plasma products, although the risk could still be quite low. We recognise that this finding will be of concern for persons with haemophilia who will be awaiting the completion of the ongoing investigations and their interpretation.

The priority is to ensure that patients are informed of this development and have access to the latest information and specialist advice from their own haemophilia centre doctor as soon as possible.

"This finding does not change our understanding of the risk from vCJD for other people in any specific way. But it does reinforce the importance of the precautionary measures that have been taken over the years.

"Since the risk of vCJD transmission through blood was first considered, a number of precautionary measures have been introduced to minimise the risk from the UK blood supply. UK plasma has not been used for the manufacture of clotting factors since 1999 and synthetic clotting factors are provided for all patients for whom they are suitable."

Ends

Notes for editors

1) The post-mortem tests were carried out as part of a research study jointly coordinated by the UK Haemophilia Centre Doctors Organisation and the National CJD Surveillance Unit. The study was commissioned in 2001 and is ongoing.

2) The likelihood of a person who is infected with the vCJD abnormal prion protein going on to develop symptoms of the disease is uncertain and may depend on individual susceptibility. It is possible that infected individuals may never develop symptoms.

3) Haemophilia is a genetic blood condition in which an essential clotting factor is either partly or completely missing. This causes a person with haemophilia to bleed for longer than normal. Treatment for haemophilia is usually by replacing the missing clotting

factor (factor VIII) through regular injections which helps the blood to clot and minimises the likelihood of long term joint damage.

4) In 2004 all patients with bleeding disorders who had been treated with UK-sourced pooled plasma products (e.g. clotting factors for individuals with haemophilia) between 1980 and 2001 were told that, owing to potential vCJD infectivity from these products, they would be classified as at-risk of vCJD for public health purposes.

The start date of 1980 is thought to be the earliest date the agent (abnormal prion protein), that causes BSE in cattle and vCJD in humans, could have entered the food chain. The end date of 2001 is the last possible expiry date of any product manufactured by UK fractionators that had been sourced from UK donors up until 1998.

5) The government introduced a number of measures from 1997 onwards to safeguard blood and plasma supplies.

- Since 1997 all cases of vCJD that are reported to the National CJD Surveillance Unit and diagnosed as having 'probable' vCJD, result in a search of the UK Blood Services blood donor records. If the patient has donated blood, any unused parts of that blood are immediately removed from stock. The fate of all used components of blood from the donor is traced, and surviving recipients informed of their risk.
- In July 1998, the Department of Health announced that plasma for the manufacture of blood products, such as clotting factors, would be obtained from non-UK sources.
- Since October 1999, white blood cells (which may carry the greatest risk of transmitting vCJD) have been removed from all blood used for transfusion.
- In August 2002 the Department of Health announced that fresh frozen plasma for treating babies and young children born after 1 January 1996 would be obtained from the USA, extended to all children under 16 years of age (Summer 2005).
- In December 2002, the Department of Health completed its purchase of the largest remaining independent US plasma collector, Life Resources Incorporated. This secures long-term supplies of non-UK blood plasma for the benefit of NHS patients.
- Since April 2004, blood donations have not been accepted from people who have themselves received a blood transfusion in the UK since 1980. This has been extended to include apheresis donors and donors who are unsure if they had previously had a blood transfusion (August 2004).
- Since late 2005, blood donations have not been accepted from donors whose blood was transfused to patients who later developed vCJD.
- The UK Blood Services continue to promote the appropriate use of blood and tissues and alternatives throughout the NHS.

6) Specialist advice and care concerning vCJD is available from:

The National CJD Surveillance Unit, based at the Western General Hospital Edinburgh: www.cjd.ed.ac.uk. The NHS National Prion Clinic, based at The Hospital for Neurology and Neurosurgery, Queen Square, London http://www.nationalprionclinic.org/

7) For further information about vCJD go to:
www.hpa.org.uk/cjd
http://www.hpa.org.uk/vcjdplasmaproducts
http://www.dh.gov.uk/PolicyAndGuidance/HealthAndSocialCareTopics/CJD/fs/en
http://www.blood.co.uk/
http://www.cjd.ed.ac.uk
http://www.nationalprionclinic.org/

8) For Health Protection Agency media enquiries please contact the Agency's Centre for Infections Press Office on:

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Last reviewed: 17 February 2009

別紙様式第4

医薬品　研究報告　調査報告書

識別番号・報告回数	F	第1報	報告日 2009年03月04日	第一報入手日 2009年02月18日	新医薬品等の区分 該当なし	機構処理欄
一般的名称	1. 乾燥濃縮人血液凝固第８因子（6343406） 2. ルリオクトコグアルファ（遺伝子組換え）（6343432） 3. 乾燥人血液凝固因子抗体迂回活性複合体（6343414）			http://www.hpa.org.uk/webw/HPAweb&HPAwebStandard/HPAweb_C/1234859690543?p=1231252394302	公表国 イギリス	
販売名（企業名）	1. ヘモフィルM（634340612）（Baxter） 2. リコネイト（634343201） 3. ファイバ（634341401） 4. ガンマガード（634342002） 5. プラズマプロテインフラクション（634342204）	研究報告の公表状況				
研究報告の概要	（概要）：イギリスのおける血友病患者でのvCJDのリスクに関する報告 HPA（英国 Health Protection Agency）から、感染に対する規制が導入される以前に血漿分画製剤を投与された70歳代の血友病患者において、後死によりvCJD感染が報告された。他の死因や症状はなかったとHPAは報告している。 この血友病患者においてvCJD異常性プリオン蛋白質がどのように感染したかについての最終の評価はまだであるが、vCJDに対する安全性を確保する法案が導入された1999年以前、この患者が、1996年に血漿を提供し６カ月後にvCJDの症状を発現したドナーの血漿から生産されたバッチの第VIII因子製剤で治療されていたことは知られている。本報告は、vCJD異常性プリオン蛋白質が、初めて血友病患者において見いだされた、あるいは血漿分画製剤を使用された患者での最初の報告である。凝固因子製剤によるvCJDの感染のリスクは、医師によって血友病患者へ伝えられており、2004年にはすでに1980から2001年の間に英国の血漿から生産された製剤で治療されていた血友病患者において、vCJD感染のリスクがあると言われていた。この新しい調査結果は、今まで理論的なリスクであったものが、リスクはまだ非常に小さいものであるが、血漿分画製剤で治療された患者にとって、実際のリスクがあることを示す。血液を通しての vCJD感染のリスクが最初に評価されていたときから、多くの予防措置が英国の血液の供給からリスクを最小にするために導入されている。英国の血漿が1999年から凝固因子製剤の製造のために使われておらず、合成された凝固因子製剤が患者に提供されている。					使用上の注意記載状況・その他参考事項等 [使用上の注意記載事項] ヘモフィルM：記載なし リコネイト：現在までに本剤の投与によりvCJD変異型クロイツフェルト・ヤコブ病（vCJD）等が伝播したとの報告はない。しかしながら、本剤の添加物である人血清アルブミンの製造工程において異常プリオンを低減し得るとの報告があるものの、理論的なvCJD等の伝播のリスクを完全には排除できないので、投与の際には患者への説明を十分行い、治療上の必要性を十分検討の上投与すること。 ファイバ、ガンマガード、プラズマプロテインフラクション、プミネート：現在までに本剤の投与により変異型クロイツフェルト・ヤコブ病（vCJD）等が伝播したとの報告はない。しかしながら、製造工程においてvCJD等の伝播のリスクを完全には排除できないので、投与の際には患者への説明を十分行い、治療上の必要性を十分検討の上投与すること。
	報告企業の意見	今後の対応				
	当該事象は、血漿分画製剤を投与された血友病患者で初めて報告されたものである。患者は、供血後にvCJDを発症したドナーから製造された血漿分画製剤を投与されていたことから、血漿分画製剤との関連を否定できないと考える。 なお、本報告で使用された製剤は、非加熱製剤の可能性が高いと考える。また、ヘモフィルMは承認を有しているが、本邦の市場には流通していない。リコネイトについても、販売を中止し本邦において、現在流通していない。ファイバ、ガンマガード、プラズマプロテインフラクション、プミネートについては、当該報告と採血国も異なり、また、これまでにvCJD感染の報告もなく、感染のリスクは低いと考える。	今後も同様の情報収集に努める。				

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別紙様式第4

医薬品　研究報告　調査報告書

識別番号・報告回数		報告日	第一報入手日	新医薬品等の区分	機構処理欄
一般的名称	4. 乾燥イオン交換樹脂処理人免疫グロブリン（6343420） 5. 加熱人血漿たん白（6343422） 6. 人血清アルブミン（6343410）	研究報告の公表状況		公表国	
販売名（企業名）	） 6. ブミネート（6343410005）				
研究報告の概要					使用上の注意記載状況・その他参考事項等
報告企業の意見		今後の対応			

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Protecting people
Preventing harm
Preparing for threats

vCJD abnormal prion protein found in a patient with haemophilia at post mortem

17 February 2009

Evidence of infection with the agent (abnormal prion protein) that causes variant Creutzfeldt-Jakob Disease (vCJD) has been found at post mortem in the spleen of a person with haemophilia.

The patient, who was over 70 years old, died of a condition unrelated to vCJD and had shown no symptoms of vCJD or any other neurological condition prior to his death. The vCJD abnormal prion protein was only identified during post mortem research tests.

The Health Protection Agency is working with the UK Haemophilia Centre Doctors Organisation to ensure all patients with bleeding disorders are made aware of this preliminary information which is being further investigated. This new finding will not change the way patients with haemophilia are cared for or treated.

A final view as to how vCJD abnormal prion protein was transmitted to this haemophilia patient has yet to be reached because investigations are continuing to determine the most likely route of transmission. It is known that the patient had been treated with several batches of UK sourced clotting factors before 1999, which is when measures to improve the safety of blood in relation to vCJD were introduced. The patient's treatment had included one batch of Factor VIII that was manufactured using plasma from a donor who went on to develop symptoms of vCJD six months after donating the plasma in 1996.

This is the first time that vCJD abnormal prion protein has been found in a patient with haemophilia, or any patient treated with plasma products. This new finding, however, does not change the public health vCJD 'at risk' status of patients with bleeding disorders.

Haemophilia patients have previously been informed by their doctors of their possible increased risk of exposure to vCJD via clotting factors. In 2004 all patients with bleeding disorders who had been treated with UK-sourced pooled plasma products between 1980 and 2001 were told that, owing to potential vCJD infectivity from these products they were to be classified as at-risk of vCJD for public health purposes.

Professor Mike Catchpole, Director of the Health Protection Agency's Centre for Infections, said:

"This new finding may indicate that what was until now a theoretical risk may be an actual risk to certain individuals who have received blood plasma products, although the risk could still be quite low. We recognise that this finding will be of concern for persons with haemophilia who will be awaiting the completion of the ongoing investigations and their interpretation.

The priority is to ensure that patients are informed of this development and have access to the latest information and specialist advice from their own haemophilia centre doctor as soon as possible.

"This finding does not change our understanding of the risk from vCJD for other people in any specific way. But it does reinforce the importance of the precautionary measures that have been taken over the years.

"Since the risk of vCJD transmission through blood was first considered, a number of precautionary measures have been introduced to minimise the risk from the UK blood supply. UK plasma has not been used for the manufacture of clotting factors since 1999 and synthetic clotting factors are provided for all patients for whom they are suitable."

Ends

Notes for editors

1) The post-mortem tests were carried out as part of a research study jointly coordinated by the UK Haemophilia Centre Doctors Organisation and the National CJD Surveillance Unit. The study was commissioned in 2001 and is ongoing.

2) The likelihood of a person who is infected with the vCJD abnormal prion protein going on to develop symptoms of the disease is uncertain and may depend on individual susceptibility. It is possible that infected individuals may never develop symptoms.

3) Haemophilia is a genetic blood condition in which an essential clotting factor is either partly or completely missing. This causes a person with haemophilia to bleed for longer than normal. Treatment for haemophilia is usually by replacing the missing clotting factor

(factor VIII) through regular injections which helps the blood to clot and minimises the likelihood of long term joint damage.

4) In 2004 all patients with bleeding disorders who had been treated with UK-sourced pooled plasma products (e.g. clotting factors for individuals with haemophilia) between 1980 and 2001 were told that, owing to potential vCJD infectivity from these products, they would be classified as at-risk of vCJD for public health purposes.

The start date of 1980 is thought to be the earliest date the agent (abnormal prion protein), that causes BSE in cattle and vCJD in humans, could have entered the food chain. The end date of 2001 is the last possible expiry date of any product manufactured by UK fractionators that had been sourced from UK donors up until 1998.

5) The government introduced a number of measures from 1997 onwards to safeguard blood and plasma supplies.

- Since 1997 all cases of vCJD that are reported to the National CJD Surveillance Unit and diagnosed as having 'probable' vCJD, result in a search of the UK Blood Services blood donor records. If the patient has donated blood, any unused parts of that blood are immediately removed from stock. The fate of all used components of blood from the donor is traced, and surviving recipients informed of their risk.
- In July 1998, the Department of Health announced that plasma for the manufacture of blood products, such as clotting factors, would be obtained from non-UK sources.
- Since October 1999, white blood cells (which may carry the greatest risk of transmitting vCJD) have been removed from all blood used for transfusion.
- In August 2002 the Department of Health announced that fresh frozen plasma for treating babies and young children born after 1 January 1996 would be obtained from the USA, extended to all children under 16 years of age (Summer 2005).
- In December 2002, the Department of Health completed its purchase of the largest remaining independent US plasma collector, Life Resources Incorporated. This secures long-term supplies of non-UK blood plasma for the benefit of NHS patients.
- Since April 2004, blood donations have not been accepted from people who have themselves received a blood transfusion in the UK since 1980. This has been extended to include apheresis donors and donors who are unsure if they had previously had a blood transfusion (August 2004).
- Since late 2005, blood donations have not been accepted from donors whose blood was transfused to patients who later developed vCJD.
- The UK Blood Services continue to promote the appropriate use of blood and tissues and alternatives throughout the NHS.

6) Specialist advice and care concerning vCJD is available from:

The National CJD Surveillance Unit, based at the Western General Hospital Edinburgh: www.cjd.ed.ac.uk. The NHS National Prion Clinic, based at The Hospital for Neurology and Neurosurgery, Queen Square, London http://www.nationalprionclinic.org/

7) For further information about vCJD go to:
www.hpa.org.uk/cjd
http://www.hpa.org.uk/vcjdplasmaproducts
http://www.dh.gov.uk/PolicyAndGuidance/HealthAndSocialCareTopics/CJD/fs/en
http://www.blood.co.uk/
http://www.cjd.ed.ac.uk
http://www.nationalprionclinic.org/

8) For Health Protection Agency media enquiries please contact the Agency's Centre for Infections Press Office on:

Kate Swan	020 8327 7097
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Louise Brown	020 8327 7080
George Fletcher	020 8327 6690

Last reviewed: 17 February 2009

別紙 3-1

医薬品
医薬部外品　研究報告　調査報告書
化粧品

識別番号・報告回数		回	報告日 年 月 日	第一報入手日 2009 年 3 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				Decontamination of prion protein (BSE301V) using a genetically engineered protease		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）		研究報告の公表状況		Dickinson, J. et al., J. Hosp. Infect. 72; 65-70, 2009		英国	BYL-2009-0371
研究報告の概要	英国健康保護局は，手術器具の汚染除去に適すると考えられる新規のプリオン不活化法を開発する試みとして，Bacillus lentus，サブチリシン遺伝子を変異させて得られたアルカリプロテアーゼである MC3（または PrionzymeTM）の使用について説明している。この研究プログラムは，散発的クロイツフェルト・ヤコブ病（CJD）が神経外科手術で伝播するという所見によって進められた。新規変異株は野生型サブチリシンのアルカリプロテアーゼ中，3 つのアミノ酸が置換されており，より高い熱安定性ならびにより速い触媒速度を示す。著者らは本稿において，プリオンに感染したマウスの脳ホモジネートを MC3 とともに pH 8，10 および 12 で in vitro でインキュベートしたところ，すべての免疫反応性 PrP^{Sc}（感染性プリオンタンパク質）が完全に分解されたことをウェスタンブロット法で確認した。同条件下においては部分的にしか分解しないプロテイナーゼ K と比較すると，MC3 はより高い有効性を示した。これらの結果は in vivo の実験によって裏付けられており，この実験では MC3 で分解した感染性マウス脳ホモジネート（iMDH）でスパイクしたマウスの 66.6%が 18 ヵ月を超えて生存し（平均インキュベート期間 447±154 日間），一方 10^{-8} に希釈した iMDH をスパイクしたマウスの生存率は 30.4%（326±162 日間）であった。この有意差は 7 log 超のプリオンクリアランスに相当する。これに対して，プロテイナーゼ K で分解した iMDH をスパイクしたマウスの全生存率は 22.7%（327±151 日）と低かった。著者らは，アルカリ条件下における MC3 による分解は，簡単かつ安全な汚染除去法であり，医療器具に対し非破壊的であることから，医療上の管理および手術器具の汚染除去において広範に適用可能であると結論付けている。				
報告企業の意見	この新規手法は，手術器具のプリオン汚染除去に関する興味深いものであり，また，適用された場合は，集団における外科的伝播のリスクならびに比較的高い有病率（英国など）を低減させるのに寄与すると考えられる。しかしながら，このような技術が血漿分画製剤の製造工程においてプリオンに対する病原体除去・不活化能力をさらに向上させることができるか否かは未だ不明である。						
今後の対応	現時点で新たな安全対策上の措置を講じる必要はないと考える。 今後，本手法の血漿分画製剤の製造工程への適用に関する情報収集に努める。						

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Journal of Hospital Infection (2009) 72, 65–70

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Decontamination of prion protein (BSE301V) using a genetically engineered protease

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Received 15 July 2008; accepted 15 December 2008
Available online 6 February 2009

KEYWORDS
Bacillus lentus subtilisin; Bovine spongiform encephalopathy; Inactivation processes; Variant Creutzfeldt–Jakob disease

Summary A previous study has demonstrated the potential of alkaline proteases to inactivate bovine spongiform encephalopathy (BSE301V). Here we explored the use of MC3, a genetically engineered variant of *Bacillus lentus* subtilisin. MC3 was used to digest BSE301V infectious mouse brain homogenate (iMBH). MC3 eliminated all detectable 6H4-immunoreactive material at pH 10 and 12; however, Proteinase K was only partially effective at pH 12. When bioassayed in VM mice, MC3- and Proteinase K-digested iMBH gave respectively 66.6% and 22.7% survival rates. Using a titration series for disease incubation, this equates to a >7 log reduction in infectivity for MC3 and >6 log reduction for Proteinase K. This study demonstrates the potential for thermostable proteases to be developed as effective inactivation processes for prion agents in healthcare management.

Introduction

The emergence of bovine spongiform encephalopathy (BSE) in cattle, believed to have given rise to variant Creutzfeldt–Jakob disease (vCJD) in humans, has generated a number of public health issues. Although any residual risk of eating BSE-contaminated food has been minimised, there continues to be a threat of onward transmission by iatrogenic routes.

Defining the levels of risk of transmission of human prion disorders via surgery, transplant or transfusion has proved difficult due to the unique nature of these agents, uncertainties about the underlying prevalence of the disease and the absence of ante-mortem diagnostic methods. The number of clinical vCJD cases remains low: 167 (dead and alive) definite or probable cases of vCJD in the UK with a further 43 cases worldwide to June 2008 [University of Edinburgh's National Creutzfeldt–Jakob Disease Surveillance Unit (NCJDSU) website]. All clinical cases have been restricted to a single genotype, being homozygous for methionine at codon 129 of the prion protein gene (*PRNP*). Further cases of potential infection, with no known clinical disease, have been identified in the two remaining genotypes: two valine homozygotes from a retrospective study looking at appendix samples and a heterozygote case of blood transfusion related transmission.[1–3] Studies suggest that clinical manifestation in these genotypes may require extended incubations and possibly present with different clinical symptoms.[4,5]

The estimation of transmission risk via vCJD is difficult as certain aspects of its presentation differ significantly from the historical forms of CJD. PrP^{Sc}, a biomarker for prion infectivity, has been found at much higher levels in vCJD-infected lymphoid and peripheral nervous tissue than sporadic CJD (sCJD) infected tissue.[6,7] This suggests that the iatrogenic transmission of vCJD is more likely than sCJD, which is supported by the identification of four cases of transfusion-related vCJD.[3,8,9] However, there are well-documented cases of transmission of sCJD via neurosurgery, transplant of dura mater and human growth hormone.[10–14] Some studies have also suggested that an increased rate of sCJD is associated with an increase in the number of surgical events an individual may have undergone.[15,16] In addition, PrP^{Sc} signal has been shown in sCJD-infected skeletal muscle and adrenal gland, suggesting that infectivity may be more widespread than originally thought.[17,18]

Given the high levels of uncertainty, validated methods for the decontamination of surgical instruments are urgently required. It is widely accepted that autoclaving only partially inactivates TSE agents.[19,20] The World Health Organization (WHO) recommends extended treatment with high concentrations of sodium hypochlorite or sodium hydroxide but these are not suitable for many applications, including routine decontamination of surgical instruments.[21] We report here an extension of our previous work using genetically engineered alkaline proteases in an inactivation model relevant to treatment of surgical instruments.

Methods

Reagents and models

Proteinase K was supplied by Finnzymes (Espoo, Finland) and MC3 ('Prionzyme™') by Danisco US Inc., Genencor Division (Rochester, NY, USA). Preparation of BSE301V mouse brain homogenate, analysis by sodium dodecyl sulphate (SDS)–polyacrylamide gel electrophoresis, western blotting and bioassays were carried out as previously described.[22] Animal studies were conducted in compliance with current UK Home Office regulations and licences.

Assessment of the subtilisin variants

The kinetic parameters k_{cat}, K_M, and k_{cat}/K_M were measured by hydrolysis of succinyl-L-Ala-L-Ala-L-Pro-L-Phe-*p*-nitroanilide (AAPFpna).[23] To assess thermal stability, purified enzyme (15 μg/mL in 0.1 mol/L glycine, 0.01% Tween-80, pH 10.0) ± 50 mmol/L $CaCl_2$ was incubated at 10 °C for 5 min, 10 °C to 60 °C over 1 min and 60 °C for 20 min, then placed on ice for 10 min and enzyme activity measured by hydrolysis as above.

Results

Protease digestion of infectious MBH (IMBH)

Digestion of BSE301V iMBH by MC3 was assessed at different pH values. At all pH values tested, MC3 was able to reduce the levels of 6H4-reactive material as detected by western blot (Figure 1). The western blots showed the presence of the characteristic triple bands corresponding to the unglycosylated, monoglycosylated and diglycosylated forms of PrP^{Sc}. Bands were fully digested at pH 8, 10 and 12: this is in line with previous results showing that alkaline pH is critical to effective digestion of PrP^{Sc} by subtilisin-type proteases.[22] At pH 4, PrP^{Sc} is only partially digested, whereas greater digestion is observed at pH 2 and 6, suggesting that the conformation at pH 4 may be especially resistant to proteolysis. Proteinase K was relatively poor at eliminating PrP^{Sc} with a smear of material evident even at pH 12, partial digestion of PrP^{Sc} at pH 8 and 10, resolution to PrP^{Sc} at pH 6 and little or no effect at pH 2 and 4 (Figure 1). Digestions of iMBH with MC3 at 50, 60, 70 and 80 °C were analysed by western blot; digestion at 60 °C appeared to be most effective (data not shown).

Assessment of MC3-digested iMBH by bioassay gave a highly significant reduction in the overall levels of infectivity compared with our previous study (Figure 2). Of the mice challenged with MC3-digested iMBH, 66.6% survived to the end of the study with a mean incubation of 447 ± 154 days (assuming that all animals had been culled at 18

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E-mail address: mark.sutton@hpa.org.uk

0195-6701/$ - see front matter
doi:10.1016/j.jhin.2008.12.007

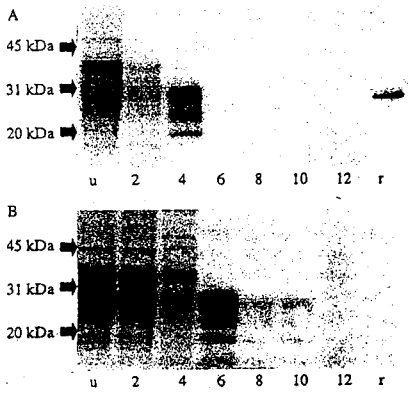

Figure 1 Degradation of 6H4-immunoreactive material by proteases under alkaline conditions. Infectious mouse brain homogenate (iMBH) dialysed against pH buffer indicated, digested for 30 min at 60 °C with 2 mg/mL MC3 (A) and Proteinase K (B). Digestion was assessed by western blot with 6H4. Lane r: recombinant PrP (Prionics); lane u: undigested iMBH in PBS.

months post challenge). This compares with 29.2% (315 ± 173 days) and 30.4% (326 ± 162 days) survival for 10^{-7} and 10^{-8} dilutions of iMBH respectively.[22] The incubation period (447 ± 154 days) is significantly different from the 10^{-8} dilution (326 ± 162 days), equating to a >7 log clearance, as assessed by Kaplan–Meier survival analysis ($P = 0.0195$). Data from our previous study show incubation of iMBH with pH 12 buffer resulted in a reduction in infectivity of ~1 log from 135.8 ± 5.59 to 142.8 ± 9.62 for an equivalent dilution of iMBH. Extended incubation periods of 327 ± 151 days were also obtained for Proteinase K digestion although there was a lower overall survival rate of 22.7%. These values were not significantly different from the 10^{-8} dilution ($P = 0.7971$). Histology was carried out to confirm disease pathology and showed complete correlation between clinical endpoint and signs of disease in the brain (results not shown).

Relevance of the genetic modifications to MC3 and its ability to digest prion material

MC3, a proprietary alkaline protease, represents a genetically engineered variant of the *Bacillus lentus* subtilisin with amino acid changes N76D/

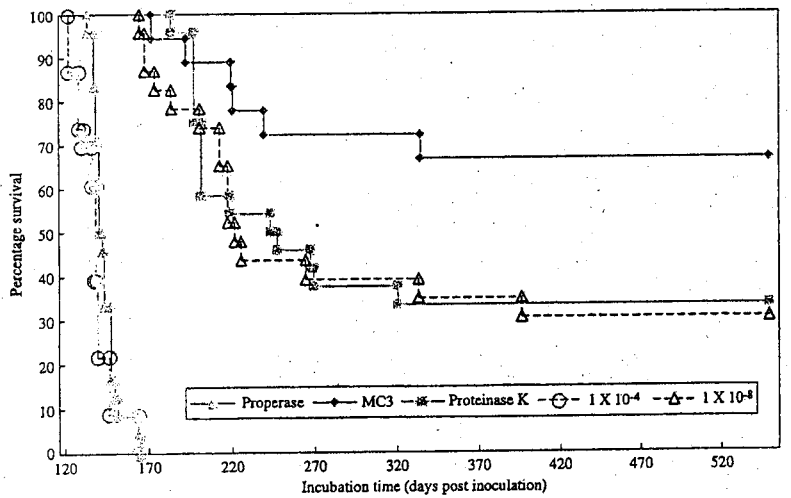

Figure 2 Bioassay survival curves of protease-treated BSE-301V infectious mouse brain homogenate (iMBH) in VM mice. Percentage survival of mice was assessed following protease digestion of 10% BSE301V iMBH. The values were compared to a titration of iMBH in naive MBH (nMBH) as published previously.[22]

Table 1 Thermal stability and enzymatic characteristics of engineered proteases[a]

	Thermal stability ($T_{1/2}$ min)[b] (% of Purafect)	Enzyme kinetic analysis		
		k_{cat} (1/s)	K_M (M)	k_{cat}/K_M
Purafect	100	170	0.78	2.20E + 05
Properase	100	435	1.89	2.30E + 05
MC3	460	830	1.6	5.20E + 05

[a] Measured by hydrolysis of succinyl-L-Ala-L-Ala-L-Pro-L-Phe-*p*-nitroanilide at pH 8.6, 25 °C.
[b] pH 10 buffer.

S103A/V104I.[24] The effects of the three amino acid modifications in MC3 were compared with both the parent enzyme (wild type *Bacillus lentus* subtilisin; Purafect) and Properase (Table I). At pH 10, MC3 shows significantly improved thermal stability with the half-life of the enzyme extended by >4.5-fold compared with Purafect and Properase. Enzyme kinetics were measured by hydrolysis of the substrate AAPFpna. MC3 showed significantly improved catalytic properties with a higher catalytic rate (k_{cat}/K_M) of 5.2×10^5 compared with 2.3×10^5 for Properase and 2.2×10^5 for Purafect.

Discussion

This study demonstrates the ability of genetically engineered proteases, selected for improved stability and catalytic properties at alkaline pH, to digest prion material. We have reduced BSE301V infectivity from ~3 log with Properase in our previous study to >7 log with MC3. This compares favourably with established decontamination/disinfection processes and is beyond the 5 log reduction recommended by the Spongiform Encephalopathy Advisory Committee (SEAC) on its website. MC3 can be easily introduced into existing healthcare practice and avoids the harsh conditions for instruments, operators and the environment that are associated with the WHO guideline conditions of 20 000 ppm active chlorine or 1 mol/L sodium hydroxide. The alkaline pH required for inactivation is comparable with other alkaline prion inactivating cleaners on the market (neodisher® Septo-Clean, Serchem Delta) and is compatible with stainless steel instruments. The 30 min contact time of the enzyme with iMBH was selected on the basis of compatibility with a pre-soak process for the final product.

Since the publication of our earlier study, several other groups have described approaches to the inactivation of prions including further protease-based, detergent-based and gas-phase inactivants.[20,25–35] Comparing data between these models is extremely difficult as a variety of TSE agents, animal models and challenge regimes have been used to assess decontamination. The relevance of different scrapie strains to the inactivation of human prion agents (and BSE) has been questioned, given their lower stability to thermal, chemical and enzymatic denaturation.[20,36,37] Given this uncertainty it would seem most appropriate to use a high titre TSE agent that is directly relevant to human disease in an animal model with no transmission barrier. At the moment such models are limited to the type of strain used here, a murine passaged BSE strain as a model for BSE and vCJD, or vCJD/sCJD in a human transgenic murine model.

Many inactivation studies have used the wire implant model as pioneered by Weissman and colleagues.[38] Although these studies are clearly directly relevant for assessing the effectiveness of prion removal from steel surfaces, they may be limited in their ability to show large reduction values due to the limited volume of material on the wire. Titration curves for 263K scrapie on wires consistently show a very rapid decrease in infectivity at ~4–6 log dilutions compared to the 8 log dilutions typical of this agent in solution.[30,31] Limited data using wire implants in overexpressing transgenic mouse models offer no improvement in sensitivity.[25,33] SEAC has highlighted on its website the need for a standardised model which can be used to compare the efficacy of decontamination technologies. The guidance suggests that a suitable model should mimic the clinical situation, for which the decontamination technology is to be used, as closely as possible. SEAC recognises that infectivity bound to a metal surface, particularly material that is dried on, may represent a tougher challenge than infectivity in solution. However, the guidance also suggests that any decontamination process should demonstrate a reduction in infectivity of ≥5 log, to offer an appreciable improvement over existing practices. In reality this may mean that two models need to be employed: a wire model to demonstrate clinical efficacy and an 'in-solution' model to ensure sufficient dynamic range.

The improved thermal stability and increased catalytic rate of MC3 have clearly contributed to its ability to inactivate prions. The ability of proteases to degrade prions appears to be dependent on the use of reaction conditions or additives that open up the structure of the infectious molecule to allow access to the peptide bonds. Based on previous results we continued to use alkaline conditions as this appeared to be generally more efficient at allowing protease digestion. This was supported by bioassay results showing a reduced log inactivation when experimentally the pH dropped below pH 12 (results not shown). Other groups have used detergent, principally SDS, usually in the presence of heat to effect similar conformational changes to promote protease digestion.[27,28,39]

There are many examples of the use of genetic engineering to enhance the properties of naturally occurring enzymes, and subtilisin-type proteases have been among the foremost of those modified.[22] Properase and MC3 are all engineered versions of the *B. lentus* subtilisin backbone and were selected for these studies on the basis of their stability and activity at alkaline pH. Clearly such an approach has applications in healthcare management with the methods being simple and safe to use and non-destructive to medical instruments.

Acknowledgements

We acknowledge the expert assistance of the Biological Investigations Group at the Centre for Emergency Preparedness and Response, Health Protection Agency. Statistical analysis was carried out by M. Vassey with input from G. Hatch. Histological assessment of brains was conducted by the Veterinary Laboratories Agency, Weybridge, UK.

Conflict of interest statement

The views expressed in the publication are those of the authors and not necessarily those of the Health Protection Agency or any other funding body.

Funding source

Danisco US Inc., Genencor Division, 200 Meridian Centre Blvd, Rochester, NY, USA.

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一般的名称	①乾燥抗 HBs 人免疫グロブリン ②ポリエチレングリコール処理抗 HBs 人免疫グロブリン		研究報告の公表状況	Lancet Neurology 2009; 8: 57-66	公表国 イギリス	
販売名 （企業名）	①ヘブスブリン（ベネシス） ②静注用ヘブスブリン－IH（ベネシス）					

研究報告の概要

[変異型クロイツフェルト・ヤコブ病の遺伝的危険因子：ゲノムワイド（全ゲノム）でのアソシエーション（関連性）研究]

＜背景＞

ヒトおよび動物のプリオン病は遺伝子の管理下にあるが、PRNP（プリオンたん白をコード化する遺伝子）以外については変異型クロイツフェルト・ヤコブ病（vCJD）の原因物質である牛海綿状脳症（BSE）プリオンに対するヒト罹患しやすさについてはほとんどわかっていない。

＜方法＞

我々は vCJD のリスクのゲノムワイドアソシエーション研究（GWAS:genome-wide association study ゲノム全体を対象とした疾病との関連性の研究）を行い、また、我々の知見の再現性確認のためにヒトプリオン病の多くのカテゴリーからのサンプル（929 サンプル）および英国（UK）とパプアニューギニアから得られたコントロールサンプル（4254 サンプル）で調べた。その UK コントロールには Wellcome Trust Case Control Consortium（WTCCC）によってジェノタイピングされたものが含まれている。我々はまた、プリオン病の臨床的表現型の遺伝的調節に関してフォローアップ分析も行い、プリオン感染マウス細胞モデル中での候補遺伝子発現を分析した。

＜調査結果＞

PRNP 遺伝子座はプリオン病のいくつかのマーカーと全てのカテゴリーを通じてリスクに強く関連していた（最も関連性が強いとされた単一の SNP［一塩基多型］ の vCJD におけるアソシエーションは $p=2.5\times10^{-17}$；vCJD においてハプロタイプアソシエーションは $p=1\times10^{-24}$）。疾病リスクへの主な寄与は PRNP 多型コドン 129 によって付与されるものではあるが、別の近傍の SNP によって vCJD のリスクの増大がもたらされた。PRNP に加えて、RARB（レチノイン酸受容体 β をコードする遺伝子）の上流に技術的にバリデートされた SNP アソシエーションが 1 つあり、それはゲノムワイドな有意性を示した（$p=1.9\times10^{-7}$）。類似のアソシエーションが医原性 CJD（iCJD）患者の小規模なサンプルで見出されたが（p=0.030）、孤発性 CJD（sCJD）やクールーでは認められなかった。培養細胞では、レチノイン酸はプリオンタンパク質の発現を調節している。我々は STMN2（SCG10 をコードする遺伝子）の上流の領域中に獲得性プリオン病とのアソシエーションを 1 つ見出し、そのアソシエーションは、vCJD（$p=5.6\times10^{-5}$）、クールー潜伏期間（p=0.017），およびクールーに対する抵抗性（$p=2.5\times10^{-4}$）であった。そのリスクジェノタイプは sCJD とは関連していなかったが発症年齢の早期化をもたらしていた。さらに、Stmn2 の発現はプリオン病のマウス細胞モデルにおいては感染後 30 分の 1 に低減していた。

報告企業の意見	今後の対応
vCJDの原因病原体であるBSEプリオンに対するヒトの感受性について遺伝子レベルでの解析を行ったことについての報告である。 血漿分画製剤は理論的なvCJD伝播リスクを完全には排除できないため、投与の際には患者への説明が必要である旨を2003年5月から添付文書に記載している。2009年2月17日、英国健康保護庁（HPA）はvCJDに感染した供血者の血漿が含まれる原料から製造された第Ⅷ因子製剤の投与経験のある血友病患者一名から、vCJD異常プリオン蛋白が検出されたと発表したが、弊社の原料血漿採取国である日本及び米国では、欧州滞在歴のある献（供）血希望者を一定の基準で除外し、また国内でのBSEの発生数も少数であるため、原料血漿中に異常型プリオン蛋白が混入するリスクは1999年以前の英国に比べて極めて低いと考える。また、製造工程においてプリオンが低減される可能性を検討するための実験を継続して進めているところである。	本報告は本剤の安全性に影響を与えないと考えるので、特段の措置はとらない。

使用上の注意記載状況・その他参考事項等

代表として静注用ヘブスブリン－IH の記載を示す。

2. 重要な基本的注意

(1) 略

1) 略

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

(30)

Genetic risk factors for variant Creutzfeldt-Jakob disease: a genome-wide association study

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Summary

Background Human and animal prion diseases are under genetic control, but apart from *PRNP* (the gene that encodes the prion protein), we understand little about human susceptibility to bovine spongiform encephalopathy (BSE) prions, the causal agent of variant Creutzfeldt-Jakob disease (vCJD).

Methods We did a genome-wide association study of the risk of vCJD and tested for replication of our findings in samples from many categories of human prion disease (929 samples) and control samples from the UK and Papua New Guinea (4254 samples), including controls in the UK who were genotyped by the Wellcome Trust Case Control Consortium. We also did follow-up analyses of the genetic control of the clinical phenotype of prion disease and analysed candidate gene expression in a mouse cellular model of prion infection.

Findings The *PRNP* locus was strongly associated with risk across several markers and all categories of prion disease (best single SNP [single nucleotide polymorphism] association in vCJD $p=2\cdot5\times10^{-17}$; best haplotypic association in vCJD $p=1\times10^{-24}$). Although the main contribution to disease risk was conferred by *PRNP* polymorphic codon 129, another nearby SNP conferred increased risk of vCJD. In addition to *PRNP*, one technically validated SNP association upstream of *RARB* (the gene that encodes retinoic acid receptor beta) had nominal genome-wide significance ($p=1\cdot9\times10^{-7}$). A similar association was found in a small sample of patients with iatrogenic CJD ($p=0\cdot030$) but not in patients with sporadic CJD (sCJD) or kuru. In cultured cells, retinoic acid regulates the expression of the prion protein. We found an association with acquired prion disease, including vCJD ($p=5\cdot6\times10^{-5}$), kuru incubation time ($p=0\cdot017$), and resistance to kuru ($p=2\cdot5\times10^{-4}$), in a region upstream of *STMN2* (the gene that encodes SCG10). The risk genotype was not associated with sCJD but conferred an earlier age of onset. Furthermore, expression of *Stmn2* was reduced 30-fold post-infection in a mouse cellular model of prion disease.

Interpretation The polymorphic codon 129 of *PRNP* was the main genetic risk factor for vCJD; however, additional candidate loci have been identified, which justifies functional analyses of these biological pathways in prion disease.

Funding The UK Medical Research Council

Introduction

Prion diseases are transmissible, fatal, neurodegenerative conditions of human beings and animals that are caused by the autocatalytic misfolding of host-encoded prion protein (PrP).[1] An epizootic prion disease, bovine spongiform encephalopathy (BSE), widely exposed the population of the UK (and, to a lesser extent, many other populations) to prion infection. The subsequent diagnosis of variant Creutzfeldt-Jakob disease (vCJD) in young British adults, and the experimental finding that this was caused by BSE-like prions,[2–4] resulted in a major public and animal health crisis.

Although the number of recorded clinical cases of vCJD to date has been small (~200) in relation to the millions of people who were potentially exposed, how many individuals were infected is unclear. The clinically silent incubation period in human beings can exceed 50 years,[5] and estimates of the prevalence of subclinical infection made on the basis of screening archived surgical specimens predicts that thousands of individuals in the UK are infected.[6] Blood transfusion seems to be an efficient route of secondary transmission[7] but no screening test to ensure the safety of blood products is yet available. Case control studies have identified no unusual occupational, dietary, or other exposure to BSE prions among patients with vCJD,[8] which suggests that genetic factors might be crucial.

A known genetic factor for susceptibility to prion disease is the common single nucleotide polymorphism (SNP) at codon 129 in *PRNP*, the gene that encodes PrP in human beings. Here, either methionine (~60% allele frequency in Europeans) or valine is encoded.[9] All patients with vCJD who have been genotyped are homozygous for methionine,[10] which represents the strongest association to date of a common genotype with any disease. Although this is a powerful effect, about a third of the exposed UK population have this genotype. An important role for other genetic loci is supported by the results of mouse quantitative trait locus studies, which have identified many regions that are not linked to *Prnp* but control the highly variable prion disease incubation periods,[11,12] including that of BSE prions.[13] The importance to public health of understanding susceptibility to BSE prion infection in human beings is therefore clear.

We undertook a genome-wide association study with 100K and 500K Affymetrix arrays with all available samples

Lancet Neurol 2009; 8: 57–66
Published Online December 12, 2008
DOI:10.1016/S1474-4422(08)70055-5
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from white British patients with vCJD (n=119) compared with our own and publicly available UK control data, which was genotyped by the Wellcome Trust Case–Control Consortium (WTCCC). Because all available vCJD samples from the UK were included in the discovery phase, we went on to compare the top-ranked SNP associations and additional SNPs at the *PRNP* locus with a large and diverse collection of patients with prion disease, including those with iatrogenic CJD (iCJD), sporadic CJD (sCJD), and kuru.

Methods

Samples

Figure 1 shows the four tiers of genotyping in the study. Samples were obtained from 119 patients with vCJD (ten patients with probable vCJD and 109 patients with definite vCJD) who were diagnosed at the National Prion Clinic (NPC), London, or the National CJD Surveillance Unit (NCJDSU), Edinburgh, between 1995 and 2005 according to established criteria. Patients who acquired iatrogenic vCJD through blood transfusion were not included in this series. All patients with vCJD were thought to have acquired the disease in the UK and were of white British ethnic origin (60% were men; mean age of disease onset was 29·8 [SD 10·9] years).

For more on the criteria see http://www.advisorybodies.doh.gov.uk/acdp/tseguidance/tseguidance_annexb.pdf

Samples were obtained from 506 patients with probable or definite sCJD diagnosed according to established criteria and from 28 patients with iCJD related to exposure to cadaver-derived growth hormone in the 1980s or earlier; these samples were obtained from the NPC or the NCJDSU or from other clinical colleagues in the UK. All patients were from the UK or elsewhere in northern Europe. Although most patients were of white British ethnic origin, and all patients of known non-white ethnic origin were excluded, this information was based on names and geographical location for some samples. 325 patients had pathologically confirmed sCJD and 181 patients had a diagnosis of probable sCJD with a high specificity according to published WHO criteria, although some of these patients might have had a neuropathological diagnosis made elsewhere.[14] Mean age of disease onset was 68·2 (SD 12·0) years for the patients with sCJD and 31·1 (6·3) years for the patients with iCJD. 50% of the samples from patients with sCJD were from men.

For WTCCC genotype data see http://www.wtccc.org.uk/info/access_to_data_samples.shtml

Before 1987, kuru surveillance was done by many different investigators; however, from 1987 to 1995 surveillance was done solely by the Kuru Surveillance Team of the Papua New Guinea Institute of Medical Research. From 1996, kuru surveillance was strengthened: a field base and basic laboratory for sample processing and storage were established in the village of Waisa in the South Fore, and a wide collection of population control samples were taken.[3] The samples from patients with kuru (n=151) were taken from young children, adolescents, and adults during the peak of the epidemic and from recent cases of kuru with long incubation times in elderly patients. The patients lived in the South Fore (n=53), North Fore (n=40), Gimi (n=3), and Keiagana (n=10) regions; linguistic group was not known in 45 patients.

Elderly women who had been exposed to kuru were defined as aged older than 50 years in 2000 and from a region that had been exposed to kuru: South Fore (n=74), North Fore (n=36), Gimi (n=13), and Keiagana (n=2). The modern-day healthy population from the exposed region was obtained by matching each elderly woman to at least two current residents of the same village who were aged less than 50 years in 2000. These mostly came from the South Fore, with some from the North Fore, and a small number of individuals from Gimi, Keiagana, and Yagaria linguistic groups, as indicated. First-degree relatives of the elderly women, identified by either genealogical data or microsatellite analysis, were excluded from these groups.

155 samples were from volunteers recruited by the Medical Research Council Prion Unit from the National Blood Service (NBS). Information was collected about their sex, age, ethnic origin, and birthplace divided into 12 regions. 90 samples genotyped with Affymetrix arrays were selected to match the vCJD collection for white British ethnic origin, birthplace (by 12 regions in UK, each region was represented in patients and controls with the same ranking), and sex (proportion of men with vCJD was 60%, and the proportion of men in the NBS controls was 57%).

A further 575 UK control samples were obtained for the replication phases of the study (730 healthy controls in total) from the NBS (95 white, random, healthy young blood donors) and from the European Collection of Cell Cultures (ECACC) human random control DNA collection (480 blood donors of known age and sex). No selection was done in the replication phase of the study. Not all control samples were genotyped for all replication studies; however, there is no reason to expect significant genetic heterogeneity in our collections of UK blood donors based on analyses of the UK population done by the WTCCC and others.[15] All UK control samples contained good quality unamplified DNA. The mean age at sampling was 38·7 (SD 10·8) years, and 51% were men. In addition, we used publicly available UK control data generated by the WTCCC. In brief, 1500 samples from the 1958 British Birth Cohort and 1500 samples from the UK Blood Service Control Group were genotyped with commercial Affymetrix 500K arrays with a Bayesian robust linear model with Mahalanobis distance (BRLMM) algorithm. We did not detect any duplicate individuals between the UK control collections nor any significant differences in allele frequency between our in-house UK control collections or those genotyped by the WTCCC.

The clinical and laboratory studies were approved by the local research ethics committee of University College London Institute of Neurology and National Hospital for Neurology and Neurosurgery and by the Medical Research Advisory Committee of the Government of Papua New Guinea. The full participation of the Papua New Guinea communities was established and maintained through discussions with village leaders, communities, families, and individuals. Most of the UK samples were obtained with written consent from patients or next of kin; however, where this was not available, for example, for archival vCJD tissue obtained at post-mortem examination, we obtained the specific approval of our local ethics committee for the use of these samples in the research.

Procedures

For the samples from patients with vCJD, genomic DNA was mostly extracted from peripheral blood, although 45 samples were extracted from brain tissue. For a few samples, whole-genome amplification, either with a φ29 protocol called multiple displacement amplification (MDA; Geneservice, Cambridge, UK; ten samples) or GenomePlex Complete Whole Genome Amplification Kit (WGA2; Sigma, UK; two samples) was necessary.

For the samples from patients with sCJD or iCJD, whole-genome amplification with either MDA in 138 samples or WGA2 in 29 samples was needed. Most of the samples from patients with sCJD and iCJD were extracted from blood, although DNA from eight samples in the iCJD group and 70 samples from the sCJD group was derived from brain tissue. 112 samples from patients with sCJD were sent as DNA to the MRC Prion Unit for analysis, most of which were extracted from blood. Genomic DNA was usually extracted from peripheral blood. PAXgene blood-derived RNA samples were also collected (Reanalytix, QIAGEN, UK).

DNA from degraded archival kuru sera was isolated by QIAamp Blood DNA minikit (QIAGEN, UK) followed by whole-genome amplification with WGA2 in all but seven samples. The validation of this process for the degraded kuru samples has been reported elsewhere.[16]

Good-quality genomic DNA extracted from blood was available for 278 of 285 (98%) healthy controls from Papua New Guinea and 122 of 125 (98%) healthy elderly women with many exposures to kuru at mortuary feasts. All control samples from Papua New Guinea were extracted from blood.

All DNA samples were checked for degradation on 1% agarose gel and stored at 50 ng/μL in low-concentration tris-EDTA buffer.

Rocky Mountain Laboratory (RML) prion-infected mouse brain homogenate (0·001%) or mock-infected brain homogenate from wild-type CD-1 mice (0·001%) was used to infect GT-1 hypothalamic neuronal cells. 5000 cells were seeded into 96-well plates and incubated with either homogenate in standard growth medium (Opti-MEM supplemented with 10% fetal calf serum and 1% penicillin/streptomycin [Invitrogen, CA, USA]). The inoculum was removed after 3 days and the cells were split 1:8. Cells were then split 1:8 twice more at intervals of 3 days. High levels of prion infectivity were confirmed with the scrapie cell assay.[17] Prion-infected and mock-infected cells were maintained in standard growth medium at 37°C in 5% CO_2. Total RNA was extracted in triplicate with the RNeasy Midi kits (QIAGEN, UK) according to the manufacturer's instructions, from prion-infected and mock-infected cells that had been grown on 10-cm-diameter plates. RNA was eluted in RNAase-free water and stored at −80°C. RNA samples adjusted to a concentration of 250 ng in 5 μL were incubated at 50°C for 30 mins with an equal volume of Glyoxyl (Ambion, Warrington, UK) loading dye containing ethidium bromide. Samples were run on a 1·5% agarose mini-gel in 1x NorthernMAx glyoxyl-based gel prep and running buffer (Ambion) at 100 mV for 90 min to check sample integrity. RNA was then sent to AROS Applied Biotechnology AS (Denmark) for the following microarray analyses according to Affymetrix standard protocols: first and second strand complementary DNA synthesis was done with the SuperScriptII System (Invitrogen) from 5 μg RNA (a minor modification was made to the protocol by using an oligo-dT primer that contained a T7 RNA polymerase promoter site); labelled antisense RNA (cRNA) was prepared with the BioArray High Yield RNA Transcript Labelling Kit with biotin-labelled CTP and UTP (Enzo Life Sciences, NY, USA) and unlabelled NTPs. Unincorporated nucleotides were removed using RNeasy columns (QIAGEN). 15 μg of cRNA was fragmented, loaded on to the Affymetrix mouse expression array 430_2.0 probe array cartridge, and hybridised for 16 h. Arrays were washed, stained in the Affymetrix fluidics station and scanned with

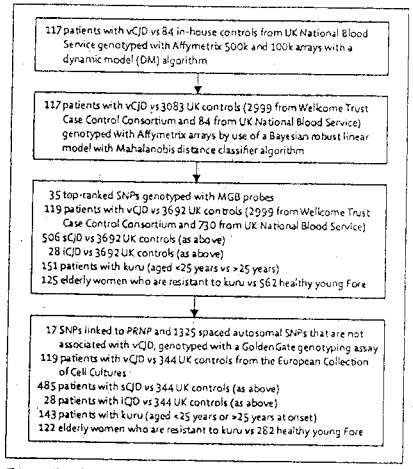

Figure 1: Flowchart of the genotyping in the tiered study
For each tier, the patient and control sample collections used are subsets of those genotypes in the minor groove-binding (MGB) probe study. In the first two tiers, the 117 samples from patients with vCJD are a subset of the 219 used in tiers three and four. In the second tier, the 84 samples from the UK National Blood Service are a subset of the 730 used in the third tier. In the final tier, the 485 samples from patients with sCJD, the 143 samples from patients with kuru, the 122 samples from elderly women, and the 282 samples from healthy young Fore are all subsets of the samples used in the third tier.

a confocal laser-scanning microscope (GeneChip Scanner 3000 System with Workstation and Autoloader).

The following sample comparisons were made in the association studies: vCJD versus UK controls genome-wide with Affymetrix array data; vCJD, sCJD, and iCJD versus UK controls in a validation and replication study with minor groove-binding [MGB] probes; healthy elderly women who were exposed to kuru at mortuary feasts versus geographically matched young individuals from the Eastern Highlands of Papua New Guinea in the replication study; young patients with kuru versus older kuru patients in the replication study. Figure 1 shows the tiered nature of the study. Subsets of each sample group have been used in previous studies of *PRNP* codon 129.[18] The comparison of young versus old patients with kuru was based on a hypothesis derived from mouse models that states that genetic factors control the incubation time of human prion diseases.[12] The incubation time of middle-aged or elderly patients who died of kuru at the peak of the epidemic cannot be calculated with precision and might have been many decades. Incubation times of up to 50 years or longer have been recorded in recently diagnosed patients,[5] whereas children, adolescents, or young adults have a limited incubation time.[3] Because the kuru collection was a mixture of samples from young and old patients, we hypothesised a priori that greater differences would be found between young people with kuru and old people with kuru than between people with kuru versus modern young healthy Fore. This strategy was supported by the precedent of homozygosity at codon 129 of *PRNP*, which was strongly associated with young versus old kuru, but was not significant in a comparison of all kuru with healthy Fore.

For the PLINK toolset see http://pngu.mgh.harvard.edu/~purcell/plink/index.shtml

Genotyping and statistical analysis

We used the Affymetrix 100K and 500K arrays (early access, EA-500K), which use four restriction enzymes in total. Our first case–control study used data generated by the Affymetrix DM (dynamic model) algorithm from 117 samples of patients with vCJD (two samples were not suitable for use with Affymetrix arrays) and 90 UK controls matched for birthplace. The 500K product is comprised of two arrays each of about 250K digested with the restriction endonucleases *NspI* or *StyI*; the 100K product is comprised of two arrays each of about 50K digested with the restriction endonucleases *XbaI* and *HindI*. Genotypes were called by the dynamic model (DM) and subsequently by BRLMM algorithms. Samples from patients with vCJD were repeated if the DM call rate was less than 85% or the BRLMM call rate was less than 90% and samples were excluded if they underperformed by these criteria (vCJD [n=0], NBS [n=6], WTCCC [n=5]). The median and mean BRLMM call rates (all non-WTCCC samples and all arrays) were 99·0% and 98·5%. No samples were excluded for excess or low heterozygosity. One duplicate sample but no related individuals were identified. With genome-wide SNP data, the PLINK toolset for whole-genome association and population-based linkage analysis enables estimates of the relatedness of individuals. For the purposes of confirming unrelatedness, this can be expressed as a probability for identity by descent (IBD)=0 using complete linkage agglomerative clustering. This probability was greater than 0·75 for all study pairwise comparisons. No samples were identified as ethnic outliers by use of identity by state clustering.

Genotype data quality analysis and filtering was done with PLINK. From 598 676 unfiltered SNPs, the following were excluded from further analysis by standard quality control: monomorphic SNPs or those not genotyped by EA-500K or WTCCC arrays (n=170 334); greater than 10% missing genotypes in vCJD (n=66 659) or WTCCC (n=9828); evidence of Hardy–Weinberg disequilibrium (exact test, $p<0·001$) in our UK samples (n=4873) or (exact test, $p<1·0\times10^{-5}$) in WTCCC samples (n=7673); minor allele frequency less than 0·01 in vCJD and WTCCC samples (n=57 853); allelic test for differences in our in-house UK samples versus WTCCC ($p<0·001$; n=1888). After this trimming, 410 287 SNPs remained for testing in 117 patients with vCJD versus 3083 UK controls (84 in-house UK controls and 2999 WTCCC samples). A more stringent filter applied additional thresholds of less than 3% missing data overall, and minor allele frequencies greater than 3% (n=288 908 SNPs remaining). These stringently filtered data were assessed for whether the skewed quartile–quartile (QQ) plots (figure 2) were caused by cryptic population stratification between the UK control and vCJD groups or alternatively by inaccurate SNP genotyping. The absence of a significantly skewed QQ plot in the stringently filtered data supports the hypothesis that SNPs were inaccurately called, probably on the EA-500K platform. Subsequently this was confirmed by concordance testing with the GoldenGate platform.

Candidate SNPs for further study were identified in stringently filtered dataset or after standard filtering if there was additional evidence of genotype accuracy, by identifying an association signal in nearby SNPs in strong linkage disequilibrium with the candidate SNP. As a further test to identify false-positive associations related to differential genotyping accuracy between cases and controls, we validated (>99% concordance) all genotypes shown in vCJD and in-house UK controls with an independent platform (MGB probe and quantitative PCR) before attempting replication in other categories of prion disease with the same technology (35 SNPs were tested in this way). To maximise coverage, a further 17 SNPs were chosen from the *PRNP* locus (by maximising pairwise r^2 with HapMap build 35 using Haploview) and genotyped with GoldenGate technology. In total 52 SNPs were genotyped for association studies further to the discovery phase.

PLINK was used for association and permutation testing. The primary analysis was an allelic χ^2 test with use of empirical p values if any cell count was less than 15. A secondary analysis implemented genotypic,

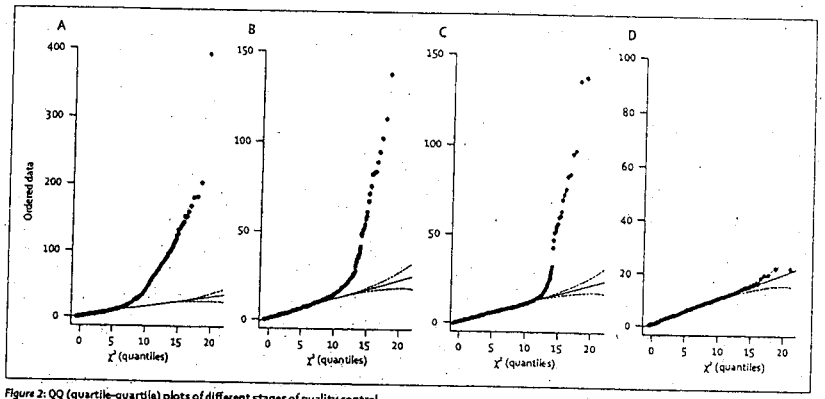

Figure 2: QQ (quartile-quartile) plots of different stages of quality control
(A) Unfiltered allelic χ^2 test of vCJD samples versus all UK samples (internal and Welcome Trust Case Control Consortium [WTCCC]) data. (B) Standard filtering allelic χ^2 test of vCJD versus all UK samples (internal and WTCCC data). (C) Standard filtering with allelic χ^2 test of vCJD versus only internal UK samples. (D) High-stringency filtering. With standard filtering, the inflation factor used for genomic control of confounding factors was estimated as 1·06 (1·01–1·09). Red dots=observed data. Blue lines=expected data. Broken blue lines=95% CI for expected data.

dominant, and recessive models, with empirical significance if necessary, controlling for the four tests done. Imputation of codon 129 genotype was done by the PLINK proxy-impute command (multimarker tagging) with dense SNP data around *PRNP*, including rs1799990, generated in 344 in-house UK controls. A nominal genome-wide significance threshold of $p<5\times10^{-7}$ was used in the primary analysis in concordance with the WTCCC. Owing to the large number of SNPs that were tested, this threshold takes into account multiple-hypothesis testing. In the replication phase of the study, the small number of tested SNPs permits a less stringent threshold of $p<0·001$.

Population structure was analysed with IBS clustering (implemented through PLINK) and principle components analysis (implemented through the Eigenstrat package[19]). Genome-wide data that were filtered to high stringency were used to compare samples from patients with vCJD and UK controls with PLINK and Eigenstrat (no significant eigenvectors were detected with default procedures). A separate low-density study was done with GoldenGate technology for several reasons: to investigate genotype accuracy in the SNPs filtered out by the high-stringency filtering step; to provide evidence with regard to the population structure in the samples from Papua New Guinea, which was previously unknown; to provide evidence that concordant genotypes consistent with the healthy population frequencies could be obtained from the amplified degraded samples from patients with kuru. 17 additional SNPs were genotyped to provide dense coverage of the *PRNP* locus, which is a region that confers susceptibility to prion disease, with a high probability of novel susceptibility being discovered here. These 17 SNPs complemented an existing dataset of 25 SNPs from the earlier genome-wide phase of the study. SNPs were selected from standard stringency filtered data in the genome-wide phase of the study; all autosomes were equally represented, with a median intermarker distance of 1·3 Mb. 1523 individuals were genotyped for 1325 SNPs: 344 randomly selected, non-related, white blood donors from the UK provided by the ECCCs; 119 patients with vCJD; 485 patients with sCJD; 28 patients with iCJD; 143 patients with kuru; 122 elderly women who are resistant to kuru and were born before 1950; and 282 young individuals from the kuru region matched to the elderly women by village of residence (figure 1). These patients were a subset of those included in the replication studies. SNPs were filtered for association with vCJD by comparison with UK controls by best permuted $p<0·001$ from any of four genetic models (allelic, trend, genotypic, or recessive) with the GoldenGate platform at the St Bartholomew's Hospital Genome Centre. Genotyping quality was assessed by Hardy–Weinberg equilibrium (excluding those assessed by exact test $p<0·001$) and visual inspection of all genotype clusters with Beadstudio version 3.1. The overall genotype call rate was 99·7%, and concordance of duplicate samples was excellent (nine WGA2 degraded amplified kuru samples [concordance 99·7%] and 20 healthy control duplicates [concordance >99·9%]). This study confirmed that the skew in the QQ plots was caused by inaccurate genotyping of SNPs in our genome-wide study that were not adequately filtered by the low-stringency criteria. For Eigenstrat, ten eigenvectors were

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	Chromosome	Locus	Minor allele	Major allele	vCJD genotypes	UK control genotypes	Model	p (vCJD)	OR
rs1799990	20	4628251	G	A	119/0/0	294/324/81	A	$2·0\times10^{-17}$	..
rs6107516	20	4625092	A	G	117/2/0	1960/1227/227	A	$2·5\times10^{-17}$	38·5 (9·6–155·2)
rs6116492	20	4646626	T	G	104/12/1	2979/104/0	A	$8·2\times10^{-7}$	3·71 (2·09–6·59)
rs1460163	8	80390003	A	G	7/25/86	31/657/2734	R	$5·6\times10^{-5}$	6·9 (3·0–16·0)
rs6794719	3	24777543	T	A	3/31/84	346/1465/1596	A	$1·9\times10^{-3}$	2·5 (1·7–3·7)

Genetic models: A=allelic; G=genotypic.

Table 1: Discovery tests of rs1799990 and four novel candidate SNPs in patients with vCJD and UK controls

	Resistance to kuru despite exposure				Early-onset and late-onset kuru				Combined p value*
	Elderly women exposed to kuru	Healthy young Fore	Model	p value	Young kuru (age <25 years)	Older kuru (age >25 years)	Model	p value (kuru incubation)	
rs1799990	16/86/23	112/287/163	G	0·001	16/18/25	9/71/12	G	$9·1\times10^{-4}$	$2·2\times10^{-7}$
rs6116492	80/37/2	393/151/18	A	0·848	47/11/0	57/20/0	A	0·44	..
rs1460163	30/77/16	140/144/40	R	$2·5\times10^{-4}$	23/29/5	26/42/23	A	0·017	$5·7\times10^{-6}$
rs6794719	5/47/63	26/118/136	A	0·12	4/25/27	7/33/47	A	0·687	..

Genetic models: A=allelic; G=genotypic; R=recessive. *Fisher's method.

Table 2: rs1799990 and three novel candidate SNPs in samples from Papua New Guinea

	iCJD genotypes	UK control genotypes	Model	p (iCJD)	sCJD genotypes	Model	p (sCJD)
rs1799990	4/13/11	294/324/81	A	$2·7\times10^{-4}$	307/98/101	G	$2·3\times10^{-21}$
rs6107516	9/12/7	1960/1227/227	R	0·002	320/100/55	G	$3·6\times10^{-13}$
rs6116492	28/0/0	2979/104/0	A	0·62	483/22/0	A	0·30
rs1460163	0/7/21	31/657/2734	A	0·66	7/93/396	A	0·83
rs6794719	1/8/19	346/1465/1596	A	0·03	61/207/207	A	0·08

Genetic models: A=allelic; G=genotypic; R=recessive.

Table 3: Replication tests of rs1799990 and four novel candidate SNPs in patients with iCJD, patients with sCJD, and UK controls

generated through default procedures and outlier detection (6 of 826 samples from Papua New Guinea were removed). No significant eigenvectors ($p>0·01$) were identified between patients with sCJD or iCJD and UK controls, or between patients with kuru, elderly women who are resistant to kuru, and healthy young Fore (five comparisons in total).

The Gene Expression Analysis Software (MAS 5.0) was used to analyse the raw image files from the quantitative scanning, which resulted in files that contained background corrected values for the probes. Significance analyses to compare prion versus mock-infected cells used a two-class unpaired test with a Benjamini–Hochberg (false discovery rate) p-value correction.

Role of the funding source

The sponsors had no role in the study design, data collection, data analysis, data interpretation, or writing of the report. Simon Mead and John Collinge had full access to all the data in the study and final responsibility for the decision to submit for publication.

Results

After standard quality control for call rate and minor allele frequency, Hardy–Weinberg disequilibrium, and differences between control datasets, we analysed 410 287 SNPs in the primary analysis. QQ plots showed an excess of large allelic χ^2 test statistics (figure 2) owing partly to the comparison between cases and controls among platforms and laboratories and was completely resolved by stringent filtering by call rate and minor allele frequency, leaving about 300K SNPs for association testing. Comparison of these stringently filtered data between vCJD and our own UK controls, ECACC controls, and WTCCC controls with Eigenstrat and GC methods did not provide evidence of significant population stratification; therefore, association statistics were not corrected. Similarly, we found no evidence of population stratification in comparisons of 1325 SNPs in replication cohorts (sCJD, iCJD, and control groups from the UK) or between patients with kuru, elderly women who were resistant to kuru, and healthy Fore from Papua New Guinea. In the stringently filtered data, two SNPs were significant at the genome-wide level ($p<5\times10^{-7}$) on the basis of allelic tests: rs6107516 in the intron of *PRNP* and rs6794719 in an intergenic region between *RARB* and *THRB*, which encodes thyroid hormone receptor beta (figure 3).

A block of linkage disequilibrium that was larger than ~100 kb and included all of *PRNP* was shown by 25 SNPs (figure 3). We added 17 more SNPs from vCJD and UK controls, including *PRNP* codon 129 (rs1799990). Unsurprisingly, rs6107516, which is located in the intron of *PRNP* and is in moderately strong linkage disequilibrium with codon 129 (rs1799990, $r^2=0·6$), was the top-ranked single SNP in the discovery phase. All patients with vCJD who have been genotyped to date are homozygous for rs1799990A (MM at *PRNP* codon 129). rs6107516 was most strongly associated in an allelic model ($p=2·5\times10^{-17}$; OR 38·5, 95% CI 9·6–155·2).

From the 25 SNPs in the discovery phase, codon 129 of *PRNP* was best tagged by a two SNP haplotype formed by rs6031692 and rs6107516 ($r^2=0·7$, based on Hapmap build 35 data) with a haplotypic association of $p=1\times10^{-24}$. To test for more association at the locus, we conditioned for the association of codon 129 by imputing this genotype and including only methionine-homozygous UK controls from the WTCCC series. We thus identified evidence of additional genetic risk at this locus. rs6116492, which is downstream of *PRNP* and also in strong linkage disequilibrium with rs1799990, had a frequency of 0·06 in patients with vCJD and 0·017 in UK controls (allelic model $p=8·2\times10^{-5}$; 0·022 in 1544 UK controls with an imputed codon 129 methionine homozygous genotype; allelic model p=0·001, OR 2·63, 95% CI 1·43–4·82). rs6116492 is located in an intergenic region between *PRNP* and *PRND*, which encodes prion-like protein doppel. Genetic risk factors for sCJD have previously been identified upstream and downstream of *PRNP* but not for vCJD.[20,21] Because we cannot guarantee that the rs1799990 genotype has been imputed perfectly, we also compared cases of vCJD (n=119) with in-house UK controls genotyped at rs1799990 and rs6116492 (n=701); we again found a significant, independent association of rs6116492, both by haplotype test conditioned on codon 129 (PLINK, likelihood ratio test with one degree of freedom; p=0·037), or simply by excluding controls with methionine/valine or valine/valine encoded at codon 129 genotypes followed by an allelic test (119 patients with vCJD *vs* 294 in-house UK controls with the genotype that encodes methionine/methionine at codon 129; p=0·02). SNP-1368 (rs1029273C, 24466 base pairs upstream of codon 129), which we and others have confirmed to be associated with sporadic CJD but not vCJD, also showed no evidence of association with vCJD independent of codon 129.[20,21]

Because we tested the entire collection of samples from white British patients with vCJD (the majority of cases of vCJD), we then looked to closely related prion diseases to replicate independently and more broadly candidate SNPs with risk of prion disease. We tested for the association of rs6794719, rs6116492, and 33 other top-ranking SNP associations from the vCJD study in patients with iCJD who were exposed to prion disease through cadaver-derived growth hormone therapy versus UK controls (n=28); 506 patients with sCJD—a worldwide disease of uniform incidence that affects about 1–2 million people per year—versus UK controls. We also tested patients with kuru and healthy elderly women who were exposed to but survived the kuru epidemic from the Eastern Highlands Province of Papua New Guinea. In the groups from Papua New Guinea, we tested whether candidates for genetic risk of vCJD were associated with kuru incubation time (by comparison with a cohort of young-onset kuru [n=59] versus old-onset kuru [n=92]) or resistance to kuru, by comparison of elderly female survivors (n=125) with the young population (n=280–526). Homozygosity at codon 129 of *PRNP* was significantly associated with risk of iCJD ($p=2·7\times10^{-4}$), sCJD ($p=2·3\times10^{-21}$), and tests done in Papua New Guinea (Fisher's method $p=2·2\times10^{-9}$; tables 1 and 2).

rs6794719A was associated with the risk of vCJD at a nominal genome-wide significance of $p=1·9\times10^{-7}$. The more frequent allele, rs6794719A, was also associated with disease risk in the small collection (n=28) of patients with iCJD (p=0·030; table 3) but not those with sCJD, kuru, or resistance to kuru.

From the 33 top-ranked SNPs that failed to achieve genome-wide significance in patients with vCJD, the strongest overall evidence of association in replication cohorts was for rs1460163 (combined $p=6·3\times10^{-8}$ by Fisher's method across orally acquired prion disease categories [combination of vCJD and Papua New Guinea

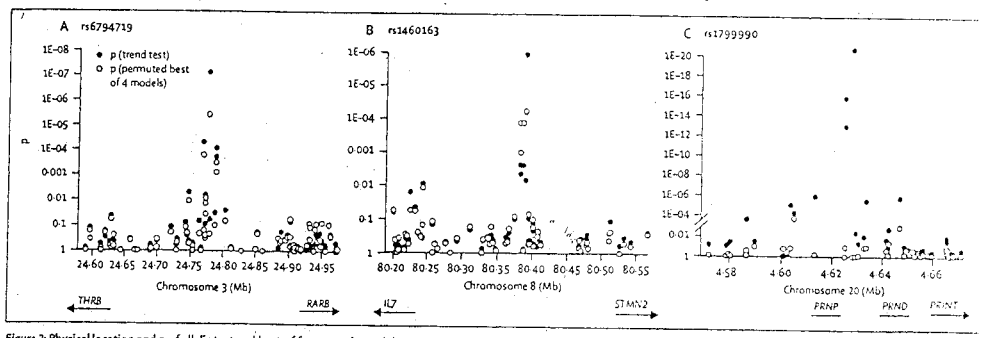

Figure 3: Physical location and p of allelic test and best of four genetic models
(A) SNPs between *THRB* and *RARB*, including rs6794719. (B) SNPs upstream of *STMN2* including rs1460163. (C) SNPs at the *PRNP* locus, including rs1799990, rs6107516, and rs6116492, showing trend test (filled circles) and a test comparing vCJD with UK controls with codon 129 methionine homozygous genotypes (empty circles [imputed for WTCCC controls]).

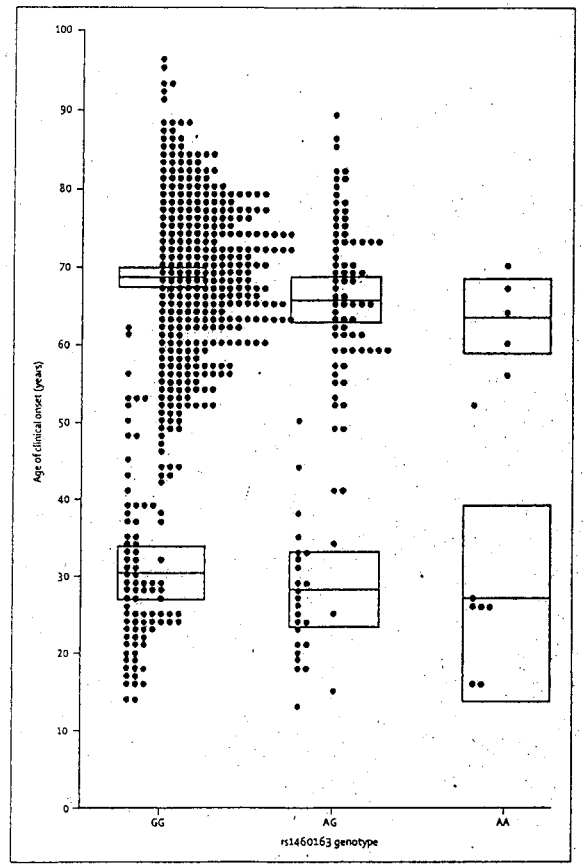

Figure 4: Age of clinical onset of vCJD (red) and sCJD (blue) patients against rs1460163 genotype
Clinical onset was defined as the age of the first symptom that progressed into a neurological or neuropsychiatric condition due to prion disease. The central bars indicate mean age of onset; boxes indicate 95% CI of the mean.

tests]). rs1460163 was associated with age of kuru onset ($p=0·017$) and resistance to kuru ($p=2·5\times10^{-4}$), with the same highest-ranking risk allele for vCJD and kuru. rs1460163 is located in a large block of linkage disequilibrium that extends just 5′ to *STMN2* (figure 3). Other SNPs tested in the replication phase were either poorly genotyped in the discovery phase (concordance <99%), or showed no evidence of association in any prion disease category additional to vCJD ($p>0·001$; best from four risk models).

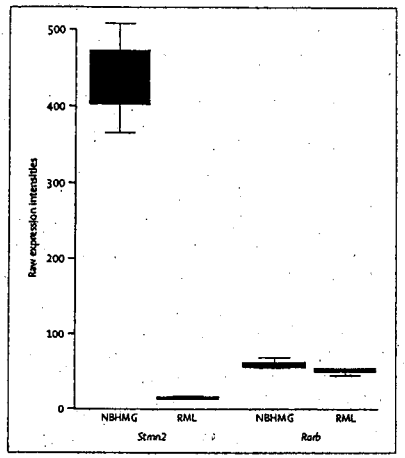

Figure 5: Boxplot of *Stmn2* and *Rarb* expression
Expression of *Stmn2* and *Rarb* in mouse neuronal cells (GT-1) treated with homogenate of healthy brain (NBHMG) or Rocky Mountain Laboratory scrapie brain homogenate (RML). Median is shown as a thick red horizontal line, IQR by boxes, and largest and smallest observations by whiskers.

We then analysed the clinical and molecular phenotype of UK prion disease for rs1460163, rs6116492, and rs6794719. In patients with sCJD, there was a significant modifying effect of the risk allele, with clinical onset 5 years earlier for those with risk genotype rs1460163AA compared with those with GG (linear regression of log-transformed age of onset against genotype $p=0·02$; figure 4). In patients with vCJD, the mean age of onset for genotype AA was 3 years earlier than for those with GG, but this was not statistically significant ($p=0·26$). By use of a linear regression model with disease type as a factor, the rs1460163AA allele was associated with age of onset in sCJD and vCJD (log-transformed $p=0·01$) and was also independent of rs1799990. No effect was seen on year of presentation, which in part will determine incubation time in vCJD, but this analysis is confounded by uncertainty in the time of exposure. No effect was seen on sCJD PrP^{Sc} strain type as defined by partial protease K digestion and western blot. rs6116492 and rs6794719 had no effect on prion disease phenotype.

In a cellular model of mouse prion disease, the expression of *Stmn2* was profoundly altered by infection with prions. This difference was shown by comparison of the transcriptome of prion-infected and prion-uninfected cells in culture. Mouse hypothalamic neuronal (GT-1) cells that were infected with mouse brain homogenate (NBHMG) or RML-infected brain homogenate were analysed with the Affymetrix Mouse Expression Array 430_2.0. Comparison of the expression between NBHMG and RML showed that *Stmn2* is significantly ($p=3·6\times10^{-18}$) downregulated by a factor of about 30 and ranked tenth out of more than 21537 genes that were represented by one or more transcripts on the array (figure 5). In this study, the expression of 543 of 21537 (2·5%) genes was altered, with a fold change of more than 2·83 (corrected Benjamini–Hochberg method). Neither *RARB* nor *STMN2* is significantly expressed in human blood cells, which obviates the analysis of the correlation of gene expression with genotypic risk in a large collection of samples.

Discussion

We describe the first genome-wide study of genetic risk in a human prion disease and replication of a small number of top-ranking candidate SNPs. Further genetic studies of human prion disease, including more extensive replication studies, are warranted because our power was limited by the small size of the vCJD sample and an early generation platform was used. Owing to the rarity of the disease, all available samples were used; the use of amplified DNA in a proportion of cases might have also affected the quality of genotyping. For these reasons, we used highly stringently filtered data and verified genotypes from candidate SNPs with an in-house assay. The potential exists for a larger scale study in sCJD that capitalises on decades of surveillance for human prion diseases across Europe and the rest of the world; however, this disease is undoubtedly more heterogeneous than vCJD.

The potential overlap in pathogenesis between vCJD and the other prion disease categories used in the replication phases of the study must also be considered. The pathogenesis of vCJD contrasts with the replication cohorts in terms of prion strain (all groups), tissue distribution, and route of infection (for iCJD and sCJD). Furthermore, in the case of our large collection from Papua New Guinea, the linkage-disequilibrium relationship between candidate SNPs and a putative functional SNP is not known and can therefore differ from that in the UK. For these reasons, an absence of association in one or more replication categories does not preclude a genuine association in vCJD.

The precedent of codon 129 was important to inform the comparisons in the replication phase. All UK prion diseases have strong associations with homozygous genotypes; for vCJD, only the methionine homozygous genotype. However, the groups from Papua New Guinea are the most relevant in the replication phase because our only precedent of a major acquired human prion disease epidemic is kuru, which was historically transmitted by cannibalism and had a devastating effect on the Fore and neighbouring linguistic groups of the Eastern Highland region of Papua New Guinea.[1] Kuru was extensively documented at its peak in the mid-20th century.[22] We amplified DNA from this archive and continued surveillance of kuru in the Fore in the late 20th century to identify recent cases of kuru with long incubation times and elderly Fore women with long-term survival after exposure to high doses of prions. At *PRNP* codon 129, elderly Fore women survivors of the kuru epidemic showed a profound Hardy–Weinberg disequilibrium, with an excess of the prion disease-resistance genotype 129MV relative to both homozygous genotypes 129MM and 129VV. The patients with kuru show an age stratification of codon 129, with young patients being mostly genotype MM or VV and adult or elderly patients being mostly MV, consistent with a powerful effect of codon 129 MV in extending kuru incubation time.[23,24] Our study thus confirms the strong association of *PRNP* codon 129 (rs1799990) across acquired and sporadic prion diseases as the outstanding genetic risk factor in human prion disease. Notably, the effect was detectable in a small sample, which should be encouraging for those contemplating studies of rare diseases with well characterised patients and a distinct pathogenesis.

The additional associations we report are not as strong or robust as those we confirm for *PRNP* codon 129 but each of these are beyond what would have been expected by chance when taking into account the problem with multiple testing. Although we cannot be certain that any of the three candidate SNPs we describe altered the expression of their nearest gene (*PRNP*, *STMN2*, or *RARB*), in each case these are excellent candidates for involvement in prion pathobiology. The risk conferred by rs6116492T could act through altered expression of *PRNP* owing to the crucial role for PrP in prion disease pathobiology; however, we have no direct evidence that a putative genetic risk conferred by rs1460163 or rs6794719 is manifest through their nearest genes (*STMN2* or *RARB*) because these SNPs have no linkage disequilibrium with coding regions. Regulatory regions often act on nearby genes but can also act over great distances or even on different chromosomes, implicating other genes.[25]

In the absence of further cohorts of orally acquired prion disease and taking into account the aforementioned caveats, we turn to functional evidence of a role for these candidate genes in prion disease. The expression of PrP in cultured neuronal and lymphoid cells is regulated by retinoic acid.[26–28] Furthermore, the production of the disease-associated isoform of PrP (designated PrP^{res}) in cultured mouse neuronal cells infected with mouse prions is increased by treatment with retinoic acid.[26] Whether retinoic acid acts through the receptor encoded by *RARB* or another retinoic acid receptor for these biological activities is not known at present. In addition to *PRNP*, the strongest overall genetic evidence we found is for a SNP association upstream of *STMN2*. SCG10, the protein product of this gene, is a regulator of microtubule stability in neuronal cells, with potential implications for aggresome formation and modulation of prion neurotoxicity.[29] We found that *Stmn2* is turned off by prion infection in mouse neuronal cells, in keeping with an early study,[30] but different from a recent and rigorously conducted study.[31] Whether prion infection or unknown experimental factors are responsible for this large effect is unclear; a role for SCG10 in prion infection has not

been established and speculation about a mechanism in prion disease would be premature.

Our data lend considerable support to the hypothesis that genetic susceptibility in addition to *PRNP* codon 129 genotype has contributed significantly to the outbreak of vCJD to date. Whether these effects are on the incubation period rather than susceptibility, such that further waves of BSE-associated prion disease with longer incubation periods might occur in the years ahead and be associated with different genotypes at many risk loci, is unknown.[32]

Contributors

SM assessed patients, conceived and designed the study, managed the data acquisition, undertook the quality control and some statistical analyses, and drafted the manuscript. MP, JU, and JB were involved in the design and conduct of the array and replication studies. GA and TW were involved in design and conduct of the MGB probe replication study. PD and SJT were involved in the design and conduct of the mouse cell expression work. JW and MPA did the Papua New Guinea field work and commented on the manuscript. CV and JCW advised on and conducted statistical analyses and commented on the manuscript. JC assessed patients, established the study and sample collections, provided overall direction, and finalised the manuscript. HH provided bioinformatics and database support.

Conflicts of interest

J Collinge is a director and shareholder for D-Gen, a company in the field of prion diagnosis, therapeutics, and decontamination. The other authors have no conflicts of interest.

Acknowledgments

This study would not have been possible without the generous support of patients, their families and carers, UK neurologists and other referring physicians, and co-workers at the National Prion Clinic, our colleagues at the National Creutzfeldt–Jakob Disease Surveillance Unit, Edinburgh, and the kuru-affected communities in Papua New Guinea. We thank the Medical Research Council/Papua New Guinea Institute of Medical Research team of local kuru reporters, including Auyana Winagaiya, Anua Senagaiya, Igana Aresagu, Kabina Yaraki, Anderson Puwa, David Pako, Henry Pako, Pibi Auyana, Jolam Ove, Jack Kosinto, Dasta Hutu, James Kisava, Sena Anua, and David Ikabala. We are grateful to Anthony Jackson, Peter Siba, John Reeder, and other staff of the Papua New Guinea Institute of Medical Research for their support. We gratefully acknowledge the help of Carleton Gajdusek, the late Joseph Gibbs, and their associates from the Laboratory of Central Nervous System Studies of the National Institutes of Health, USA, for archiving and sharing old kuru samples. The studies were initially funded by a Wellcome Trust Principal Research Fellowship in the Clinical Sciences to J Collinge, and since 2001 by the Medical Research Council. Some of this work was undertaken at University College London Hospital, which received a proportion of funding from the Department of Health's NIHR Biomedical Research Centres funding scheme.

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別紙 3-2

医薬品
医薬部外品　研究報告　調査報告書
化粧品

識別番号・報告回数		回	報告日 年　月　日	第一報入手日 2008 年 12 月 24 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				A vCJD blood test achieves 100% accuracy www.News-Medical.Net, 22 Dec 2008	公表国 オーストラリア	
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-2009-0366
研究報告の概要	昨年 12 月，Amorfix Life Sciences 社（カナダ）は異型クロイツフェルト・ヤコブ病(vCJD)のプリオンについて検討したヒト血液検体に関する第 2 回目の盲検試験の結果を公表した。当該試験は英国で実施された。同社は，多量の正常タンパク質がある検体中において非常に低レベルの凝集した異常折りたたみタンパク質を選択的に検出することができる，独自の特許権をもつ Epitope Protection™技術を開発した（詳細については，www.amorfix.com を参照のこと）。本試験の結果から，当該検査は 100%の感度および 100%の特異性を有すると主張している。試験では新鮮血漿および凍結血漿検体を用い，その一部に脳由来の vCJD プリオンを添加した。実に，脳ホモジネートを $1/10^6$ まで希釈したものを添加した検体を検出することに成功した。この技術は，vCJD 感染した集団を対象とした血漿を見極めるための大規模な検査にも適用できる。これらの結果は，以前は疾患に対し抵抗性を示すと考えられていた MV 遺伝子型をもつ人における vCJD の発症が，最近，初めて報告されたことを考慮すると，特に重要である。また，この検査法は明らかに献血および血液製剤の安全性を高めるのに役立つと考えられる。					
	報告企業の意見		今後の対応			
	この検査法はプリオン検出の向上をもたらす可能性がある。輸血用血液の安全性を高めるために有用であることが証明された場合，血漿分画製剤に用いる血漿プールにも使用される可能性がある。 弊社の血漿分画製剤の製造工程におけるプリオン除去能は 4 log を上回ることが確認されており，弊社製剤による vCJD 感染リスクは極めて低いと考えられる。		現時点で新たな安全対策上の措置を講じる必要はないと考える。 今後も Amorfix の輸血および血漿分画製剤のスクリーニングに関する情報収集に努める。			

(31)

BYL-2009-0366

vCJD blood test achieves 100% accuracy

Published: Monday, 22-Dec-2008

Disease/Infection News

Amorfix Life Sciences has announced it achieved 100% sensitivity and 100% specificity in a second blinded trial of human blood samples using its EP-vCJD blood test in collaboration with the National Institute for Biological Standards and Control (NIBSC) in the United Kingdom.

"We have now successfully completed both fresh and frozen human plasma testing, as part of a test validation process facilitated by NIBSC," said Dr. George Adams, Chief Executive Officer of Amorfix. "The company has 50,000 test kits available to begin large-scale testing to determine the fraction of the population infected with vCJD. This information is vital for determining the need for routine testing of blood donations."

The UK Spongiform Encephalopathy Advisory Committee (SEAC) yesterday announced the first clinical case of vCJD in a patient with an MV genotype (all previous vCJD clinical cases were MM genotype) and suggested that 50 to 250 further cases might arise in the UK. This is consistent with a recent editorial in a leading medical journal, Lancet Neurology, published last week suggesting "waves" of vCJD cases could be expected.

"This first MV case of vCJD now shows people with MV genotypes are not resistant to vCJD, but may incubate the disease for a longer time before developing neurological symptoms. Yesterday's report of vCJD with MV genetics shows we are not out of the woods with this tragic epidemic, and also raises the possibility of ongoing blood-borne transmission of vCJD from silent carriers of the infection," said

Dr. Neil Cashman, Chief Science Officer of Amorfix.

In the most recent panel, NIBSC provided Amorfix with 500 frozen blinded human plasma samples which included some samples spiked with vCJD brain prions. The EP-vCJD(TM) test successfully detected all (100% sensitivity) of the spiked samples down to a 1 in 100,000 dilution of 10% brain homogenate (1/1,000,000 dilution of vCJD brain). The test scored one sample initially positive (initial reactivity of 99.8%) but upon repeat testing correctly identified the sample as negative (specificity of 100%). In the first blinded panel, Amorfix tested 1,000 fresh UK plasma samples with identical perfect results.

http://www.amorfix.com

別紙様式第2-1

No. 5

医薬品 研究報告 調査報告書

識別番号・報告回数			報告日	第一報入手日	新医薬品等の区分	総合機構処理欄
				2008. 11. 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	Dorsey K, Zou S, Schonberger L, Fang C, Dodd R. TRANSFUSION 2008-Vol. 48 Supplement	公表国 米国	
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)					

研究報告の概要

○古典的CJDの輸血伝播リスクは、仮にあるとしてもvCJDよりも有意に低い－USルックバック試験

背景:1995年に米国赤十字(ARC)と疾病管理予防センター(CDC)は、古典的クロイツフェルト・ヤコブ病(CJD)についてのルックバック調査による評価を開始した。これまで、ヒトにおける古典的CJDの輸血伝播の報告はない一方、変異型CJD(vCJD)の輸血伝播は英国で報告されている。

方法:供血後にCJDと診断された供血者(CJD供血者)に由来する血液成分の受血者を登録した。生存受血者については登録以降毎年バイタルサインをモニターした。受血者が死亡した場合は死因を調査し、2005年末までの死亡を網羅した。

結果:古典的CJDを発症した供血者計35名(2名を除き孤発性CJD)および受血者430名を本試験に登録した。2005年までに生存受血者88名(1,135人年)、死亡受血者326名(813.5人年)、後に追跡不能となった受血者16名(64.5人年)の合計2,013人年の輸血後追跡調査が行われた。受血者のうち、144名は5年以上生存(長期生存者)し、CJDによる死亡は確認されなかった。長期生存者については、さらに関係する製剤輸血日と供血者のCJD診断日の間隔を調査し、英国におけるvCJDの観察と比較した。CJDの輸血伝播リスクは、vCJDと比べて有意に低かった(p = 0.0117, Fisher の直接確率検定)。

結論:今回のルックバック検査の結果は、孤発性CJDの受血者への輸血伝播の証拠がないことを示しており、CJDの輸血伝播のリスクは(仮にあったとしても)vCJDと比較して有意に低いことを示すものである。

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

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

古典的CJDを発症した供血者計35名に由来する血液成分の受血者430名のルックバック調査の結果、孤発性CJDが輸血で伝播する証拠はなく、リスクはvCJDと比較して有意に低いとの報告である。

今後の対応

これまでの疫学研究等では、血液製剤を介して古典的CJD(孤発性、遺伝性および医原性CJD)が伝播するという証拠はない。またCJDの病原因子とされる異常プリオンがアルブミン製剤の製造工程で効果的に除去されるとの報告もあるが、輸血あるいは第Ⅷ因子製剤によりvCJDに感染する可能性が示唆されたことから、今後も引き続き情報の収集に努める。なお、日本赤十字社は、CJD、vCJDの血液を介する感染防止の目的から、献血時に過去の海外渡航歴(旅行及び居住)、CJDの既往歴(本人、血縁者)、hGH製剤投与の有無を確認し、該当するドナーを無期限に献血延期としている。

MedDRA / J Ver.11.0 J

JRC2008T-075

Disclosure of Conflict of Interest
Shadaba Asad, Leonard Mermel, Joseph Sweeney, Nothing to Disclose

S86-030K

Direct Assessment of CMV Transfusion Transmitted Risks Post Universal Leukoreduction

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Background: CMV transmission through transfusion remains a clinical concern. Two effective strategies to reduce the risks of CMV TTD have been the provision of CMV seronegative blood and leukoreduction. Even though it is still under debate, these two methods have been considered operationally equivalent in many institutional policies. For many hospitals, universal leukoreduction has become the main strategy for the prevention of CMV TTD. Direct assessment of CMV TTD risk is lacking in the era of universal leukoreduction. In this study, through prospective clinical follow-up and testing of transfusion recipients (TR) for CMV Ab and nucleic acids and CMV Ab testing of their linked donors, the risk for CMV TTD was studied.

Methods: As part of a prospective study of multiple donor exposure TRs, CMV TTD risk was assessed. Transfused units were all leukoreduced and not prospectively screened to be CMV seronegative. CMV total Ab and Nucleic Acid testing (NAT) were performed on all TRs baseline samples. For TR with negative baseline CMV testing, all follow-up TR samples were tested for CMV total Ab and NAT, and retained linked donor samples were tested for CMV total Ab. In cases when CMV TTD was suspected based on seroconversion, with or without supportive clinical evidence, donors were also tested for CMV NAT when possible. Evaluable transfusion was defined as a transfusion with TR sample(s) collected 14 to 180 days post transfusion in TR with a negative baseline CMV testing. **Results:** 46 evaluable TRs were negative for CMV at baseline. There were 1319 evaluable cellular blood component transfusions. Out of these, there were 655 RBCs for 43 TRs and 664 platelets for 31 TRs. Out of 1319 retained linked donor samples, 485 were positive for CMV total Ab. There were 19 case investigations due to changes in CMV testing results. Three appeared to be true infections or seroconversion. These may be related to transfusion; however, there was no definitive proof from donor follow-up that they were transfusion associated. Two were determined to be true infections but not transfusion related. Six were attributed to passive Ab transfer. Eight could not be determined due to inadequate information. **Conclusion:** Based on the No. of infections or seroconversions over the No. of TRs who were seronegative at baseline, the calculated CMV potential TTD rate was as high as 6.5% (3/46). Based on the No. of infection or seroconversions over the No. of transfused donor units, the calculated CMV potential TTD risks was: for leukoreduced but non-CMV screened cellular products, as high as 0.23% (3/1319); for leukoreduced and CMV seronegative (tested after transfusion) leukoreduced but non-CMV screened cellular products, as high as 0.62% (3/485). In summary, post-universal leukoreduction, CMV-transfusion-transmission, while uncommon, may still occur.

Disclosure of Conflict of Interest
Richard Cable, Shiman Zou, Kem Dorsey, Yanlin Tang, Cheryl Hapip, Russell Meimet, Jonathan Trouem-Trend, Chyang Fang, Melanie Champion, Roger Dodd: Nothing to Disclose
YanYun Wu: ARC - Grants or Research Support

S87-030K

A Linked Donor and Recipient Study of B19 Viral Transmission by Blood Component Transfusion

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Background: B19 virus (B19V) transmission by pooled plasma products has occurred from Factor VIII when DNA concentration (conc) exceeded 10^4 IU/mL and from SD plasma at a DNA conc of $>10^2$ IU/mL; this has led to "in process" screening of recovered and source plasma units for high B19V DNA conc (~10^4 IU/mL). Although several cases of B19V infection have occurred from component (cmpt) transfusion (tx), B19 screening is generally not performed for transfusable components as there has been no systematic study of this transmission rate or its association with B19 DNA conc. **Methods:** DNA positive (pos) donations (dnns) were identified by screening frozen repository plasma specimens (spms) using a previously validated sensitive kinetic PCR assay. Tx transmission was evaluated using various B19V assays to test pre- and 6-12 months post-tx specimens from adult surgical patients who received B19 DNA pos and negative (neg) control components; controls were selected in a 2:1 ratio and matched for factors influencing B19 transmission by non-tx routes. B19 transmission was inferred if either DNA, IgG, or IgM seroconversion occurred post-tx as demonstrated by replicate testing. Rates were determined in susceptible (susc) (i.e. pre-tx IgG antibody (Ab) neg) recipients and controls. **Results:** 105 of 12,529 donor specimens (0.83%) were B19 DNA pos; see the Table for plasma DNA concs. 110 components (mostly rbcs) from 103 pos donations with DNA <10^4 IU/mL (all of which were IgG Ab pos) were transfused to 105 recipients. There was no evidence of B19 infection in 21 susc case recipients (rate = 0.0%; 95% CI: 0.0-13.3) or in 43 susc control recipients (rate = 0.0%; 95% CI: 0.0-8.7). The estimated tx-transmission rate was thus 0.0% (95% CI: 0.0-13.3). Two components with >10^4 IU/mL were transfused to pre-tx B19 IgG Ab pos recipients, precluding assessment of tx transmission. **Conclusions:** Although we documented a high frequency of low conc (especially <10^4 IU/mL) B19 DNA and IgG Ab pos donations, the lack of tx transmission by these components indicates that such transmission does not occur, or if it occurs, is relatively uncommon (≤13%). These data do not support the need for real time screening of blood components with a sensitive B19 NAT assay.

TABLE. Linkage of B19 DNA pos components to recipients

B19 DNA conc (IU/mL)	# pos dnns	# DNA pos cmpts tx'd to susc recipients	# DNA pos cmpts tx'd to non-susc recipients	Total # DNA pos cmpts tx'd
<20	56	15	45	60
20-<10[2]	19	2	18	20
10^2-<10^3	23	3	22	25
10^3-<10^4	4	1	3	4
10^4-<10^5	0	0	0	0
10^5-<10^6	1	0	1	1
Subtotal	103	21	89*	110*
≥10^6	2	0	2	2
Total	105	21	91*	112*

*More than one component was tx'd for some pos donations; some recipients received more than 1 pos component.

Disclosure of Conflict of Interest
Steven Kleinman, Simone Glynn, Tzong-Hsie Lee, Leslie Tobler, Karen Schlumpf, Deborah Todd, for the NHLBI Retrovirus Epid Donor Study-II, Michael P. Busch: Nothing to Disclose

S88-030K

The Risk of Transfusion Transmission of Classic CJD is Lower Than vCJD, If not Zero – Results from US Look-Back Study

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Background: In 1995, the American Red Cross (ARC) and the Centers for Disease Control and Prevention (CDC) initiated a look-back investigation to assess the risk of transfusion transmission of classic forms of Creutzfeldt – Jakob disease (CJD), sporadic, familial and iatrogenic CJD. The presence of the infectious agent of classic CJD in blood has been documented in experimental animals, but no transfusion transmission of classic CJD in humans has been reported. In contrast, transfusion transmission of variant CJD (vCJD) has been documented in the United Kingdom. **Methods:** Blood donors who were subsequently diagnosed as having CJD (CJD donors) were identified by reports primarily from collaborating blood centers, the Food and Drug Administration (FDA), and family members. Following verification that the CJD diagnosis was made by a neurologist or confirmed by a pathologist, blood centers identified the donations made by the CJD donor and located the hospitals that received any of the blood components. The recipients of these components that were identified by hospitals were enrolled into the study. Their vital status, and cause of death, if deceased, was monitored by searching CDC's National Death Index (NDI) database at enrollment and every year thereafter for surviving recipients. The last NDI search covered deaths through the end of 2005. **Results:** A total of 35 blood donors with classic (non-variant) CJD and 430 recipients were enrolled in the study. All but 2 donors had sporadic CJD. Through 2005, recipients contributed a total of 2,013 person-years (py) of follow-up time since receipt of their blood transfusion. 1,135 py from 88 surviving recipients, 813.5 py

from 326 deceased recipients and 64.5 py from 16 recipients who were subsequently lost to follow up. Among the recipients, 144 survived 5 years or more (long-term survivors). No deaths from CJD were identified among the recipients. The most common causes of death among the recipients were cancers (followed by cardiovascular diseases. The long-term survivors were further analyzed by the interval between the date of transfusion of the implicated unit and the date of diagnosis of CJD in the donor. A comparison of observations of vCJD in the UK (TMER study) using recipients who lived 5 or more years post-transfusion and had received the unit of blood from a donor whose symptoms occurred 60 months prior to onset of symptoms. The transfusion transmission risk of CJD was statistically significantly lower than that of vCJD ($p = 0.0117$, Fishere exact test). Conclusions: The results from this look-back study continue to show no evidence of transfusion transmission of sporadic CJD to recipients. The results indicate that the risk, if any, of transfusion transmission of CJD is significantly lower that than that of vCJD.

Disclosure of Conflict of Interest
Kerri Dorsey, Shimian Zou, Lawrence Schonberger, Chyang Fang, Roger Dodd: Nothing to Disclose

S89-030K
Current Value of Serologic Test for Syphilis as a Surrogate Marker for Bloodborne Viral Infections among US Blood Donors
S Zou[1] (Dodd@usa.redcross.org), E Notari[2], C Fang[1], S L Stramer[3], R Dodd[1], *[1]American Red Cross, Rockville, MD; [2]American Red Cross Blood Services Holland Lab, Rockville, MD; [3]American Red Cross Biomedical Services, Gaithersburg, MD; [4]American Red Cross Blood Services, Rockville, MD.*

Background: Routine serologic screening for syphilis has been conducted for two reasons: 1) preventing transfusion transmission of syphilis although its value has been shown to be limited; 2) serving as a surrogate marker for infections caused by other pathogens such as HIV. This study assessed the current value of the test in safeguarding the blood supply against other presently tested viral infections. **Methods:** Testing results for voluntary and directed, whole blood, repeat donors in 2005-2006 with a large US blood supplier were analyzed. All donations were tested according to standard procedures for anti-HIV, HIV RNA, anti-HCV, HCV RNA, HBsAg, antibodies against human T-lymphotropic virus (anti-HTLV), syphilis and other markers. Incidence rate was defined as the number of confirmed seroconverters or viral RNA converters over total number of person-years observed. Sensitivity referred to the number of confirmed syphilis seroconverters among all confirmed converters for another infection, such as HIV, whereas positive predictive value (PPV) was the number of confirmed HIV converters among all confirmed syphilis seroconverters. Applying the sensitivity of syphilis testing in identifying new infections of another agent to the estimated number of donors during the window-period of the other infection gave the estimated number of window-period (w-p) units removed by syphilis testing. **Results:** There were significantly higher frequencies of HIV, HCV, HBsAg and HTLV positive donations among those with positive syphilis tests although such samples would not be expected to escape detection because of the use of multiple tests for most markers. Among 3,068,320 repeat donors with confirmed testing for both syphilis and anti-HIV, 168 seroconverted for syphilis but not for anti-HIV, 57 seroconverted for anti-HIV but not for syphilis, with only 1 seroconverted for both, resulting in an incidence ratio of 331 (95% CI: 46, 2390) between syphilis seroconverters vs. non-seroconverters, a sensitivity of 1.7% and a PPV of 0.6%. There were 4 additional HIV RNA converters who did not seroconvert for syphilis, resulting in an incidence ratio of 309 (43, 2231), a sensitivity of 1.8% and a PPV of 0.6%. No syphilis seroconverters converted for HCV, HBsAg or anti-HTLV. The result for HIV represented an estimated removal by syphilis testing of 0.11 w-p units out of a total of 9.5 million repeat donations during the two-year period if only anti-HIV is tested and of 0.07 w-p units if HIV RNA is also tested. **Conclusion:** Our data show that syphilis testing has a very low sensitivity and PPV for detecting other viral infections. It presents no surrogate value for HCV, HBV and HTLV infections and could only remove approximately one HIV window-period unit out of every 95 million donations from repeat donors.

Disclosure of Conflict of Interest
Shimian Zou, Edward Notari, Chyang Fang, Susan L. Stramer, Roger Dodd: Nothing to Disclose

Transfusion Practice/Clinical Case Studies

S90-030L
Accumulation of Thrombospondin-1 (TSP-1) in Packed Red Blood Cells is Reduced by Whole Blood Leukoreduction Filters
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Background: Independent of other factors related to shock, blood transfusion is a risk factor for multiorgan failure (MOF) in trauma. The risk of organ failure, sepsis, and death correlates with increasing transfusion amount. Pathological specimens of organs of patients with MOF and disseminated intravascular coagulation (DIC) show widespread microvascular thrombosis. Multiple hemostatic changes occur in MOF leading to DIC. Degranulation of platelet α-granule glycoproteins correlates with the severity of MOF. TSP-1(thrombin sensitive protein) is one of the most abundant proteins in the platelet α-granule comprising 25%, and is part of a class of extracellular proteins which modulate cell-matrix interactions. TSP-1 is produced by platelets, megakaryocytes, endothelial cells, fibroblasts, monocytes, macrophages, and osteoblasts. TSP-1 binds to extracellular molecules including heparin, collagen, fibrinogen, fibronectin, plasminogen activator, and osteonectin. TSP-1 also binds to cells via CD36, CD47, and integrins. TSP-1 plays a role in platelet adhesion with other cells and with extracellular proteins. The accumulation of TSP-1 in PRBC was evaluated during PRBC storage as a possible contributor to MOF due to transfusion. **Methods:** Ten units of whole blood were separated into equal aliquots, one filtered with whole blood leukoreduction filters (Leukotrap-SC RC, Pall) and one unfiltered. Half units of PRBC were prepared and stored at 4°C in CPD-AS3. Aliquots from each unit were removed weekly, and plasma was separated from the cellular component and frozen at –80°C for simultaneous testing of PRBC supernatant for TSP-1 by ELISA (Pierce Searchlight). **Results:** During storage TSP-1 levels in PRBC supernatant increased to 24 times that seen on the day of collection. Filtration at collection results in maintenance of day 0 levels throughout storage. **Conclusions:** RBCs adhere to endothelium as they age. In trauma, with massive transfusion of old RBCs, the RE system is overwhelmed by rapid infusion of PS expressing cells which can occlude small vessels. The presence of molecules in PRBC supernatant which can lead to adhesion of platelets and leukocytes with further occlusion may contribute to development of MOF. This is especially important in the face of ongoing endothelial injury. The use of leukoreduction filters at collection prevents accumulation of TSP-1 during PRBC storage keeping levels similar to those in fresh PRBC units.

Day	TSP-1 pg/mL Filtered	SD	Unfiltered	SD
0	102,433	70,504	732,522	431,231
7	215,484	166,957	2,078,779	1,880,785
14	458,247	394,759	8,208,880	5,229,551
21	508,733	425,944	17,884,850	6,446,864
28	646,694	647,201	11,887,478	7,368,030
35	837,582	841,212	11,719,325	3,082,931
42	773,083	663,248	11,791,379	6,323,265

Disclosure of Conflict of Interest
Lisa Cardo, Donna Wilder: Nothing to Disclose

S91-030L
Does Leukoreduction of Blood Products Influence Outcomes? An Analysis of 842,738 Hospital Inpatients
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Background: Allogeneic leukocytes have been implicated as a cause of transfusion-related immunomodulation (TRIM) and have been associated with post-operative infection (POI) and systemic inflammatory response syndrome (SIRS) in transfused patients. Universal leukoreduction (LR) of red blood cell (RBC) units has been proposed as a means of eliminating TRIM and improving outcomes but the success of this strategy remains uncertain. We performed a retrospective analysis of 842,738 hospitalized patients from 20 hospitals treated between 2002 and 2007 included in our database (COMPARE™, Infonale/Haemonetics®, Braintree, MA) to determine the influence of LR on the incidence of POI and SIRS in hospitalized patients. **Methods:** Primary diagnosis, age, gender, race, emergency admission, any surgery, co-morbidities, severity of illness, any RBC, only LR RBCs (LR), any NON LR RBCs (NON LR), and NON LR RBCs plus

別紙様式第2

別紙(2)-3

医薬品　研究報告　調査報告書

識別番号・報告回数			報告日	第一報入手日	新医薬品等の区分	厚生労働省処理欄
				2009年3月30日	該当なし	
一般的名称	乾燥 pH4 処理人免疫グロブリン		研究報告の公表状況	A recipient of immunoglobulin from a donor who developed vCJD Vox Sanguinis April 1, 2009, 96/3 (270)	公表国 英国	
販売名（企業名）	①サングロボール ②サングロボール点滴静注用 2.5g （CSL ベーリング株式会社）					

研究報告の概要

問題点（vCJD に感染した供血者のプリオン伝播のリスク）
10 年間再発性の呼吸器感染に罹患していた 61 歳女性が分類不能型免疫不全症（CVID）と診断された。抗体欠乏の診断が遅延することは、残念ながら稀なことではない。患者は 1995 年から静注用人免疫グロブリン（商品名 Vigam 、BPL 社、英国）で週 3 回の治療を受けていた。1997 年 1 月から 1998 年 2 月の期間に当該患者は、後に vCJD を発症した供血者の血漿を含有した静注用人免疫グロブリンを投与された。患者は 5g 製剤 8V（バッチナンバーVGD049）、2.5g 製剤 4V（バッチナンバーVGD050）を投与された。これらのバッチの推定 ID50/g は、それぞれ 0.0000112 と 0.0000688 であった。患者は 72 歳で内臓の腺癌の再発のため死亡した。
国立クロイツフェルト・ヤコブ病サーベイランスユニットで検死が実施された。死亡後に防腐処置を施された。この過程が体組織中のプリオン物質の検出に影響するかは不明である。脾臓とリンパ節中のプリオン蛋白をウエスタン・ブロット法で検査したが陰性であった。
組織学的、免疫化学的またはウエスタン・ブロット法による解析で、脳内にプリオン蛋白が存在するエビデンスは見出せなかった。
関与しているバッチによる治療と関連性の無い死亡との間隔が 9 年であったが、赤血球成分による vCJD 伝播症例の間隔（5-8.5 年）より長かった。そのため、もし異常プリオンが伝播していたならば、エビデンスが発見されるのは当然である。
vCJD を発症した供血者の血漿を含有した静注用人免疫グロブリンを投与されたが、当該患者は臨床的に vCJD を発症せず、組織病理学的及び分子技術によりプリオン蛋白が沈着しているエビデンスを発見できなかった。
現在まで赤血球成分によるプリオン伝播は 4 症例報告されているが、静注用人免疫グロブリンによるプリオン伝播の症例報告は無い。
静注用人免疫グロブリンなどのプール血漿の製品の安全性は、伝播する可能性を減少する製造方法での多数の工程により高まっている。
現在の静注用人免疫グロブリンの製造方法は $5\log_{10}$ までプリオン分子を削減できるので、たとえ供血にプリオン蛋白が含有されていても静注用人免疫グロブリンによる vCJD 伝播のリスクは低い。
静注用人免疫グロブリンによる vCJD 伝播の報告は無いが、伝播の可能性を避けるため予防的措置として 1997 年から英国の血漿は、血漿分画製剤の製造に使用されていない。
静注用人免疫グロブリンは多くの適応症があり、世界の需要は供給を上回っている。
現在、英国では静注用人免疫グロブリンの使用できる適応症が厳重に制限されている。世界の市場から英国の供給を賄うことが次第に困難になってきているため、血漿分画製剤の製造への英国血漿使用の禁止を継続すべきかどうか再調査するのが適切かもしれない。
このことから、現在の論争を巡る多くの問題点が浮かび上がってくると考える。

使用上の注意記載状況・その他参考事項等

報告企業の意見	今後の対応
これまで本剤によるvCJDが伝播した報告はない。製造工程において異常プリオンを低減し得るとの報告があるものの、理論的なvCJD等の伝播のリスクを完全には排除できないので、投与の際には患者への説明を十分行い、治療上の必要性を十分検討の上投与することを添付文書に記載し、注意喚起している。	今後とも新しい感染症に関する情報収集に努める所存である。

ABJ2009703

LETTER

Vox Sanguinis (2009) 96, 270

DOI: 10.1111/j.1423-0410.2008.01148.x

A recipient of immunoglobulin from a donor who developed vCJD

Dear Editor,

We present the case of a female patient who at the age of 61 years was diagnosed with common variable immunodeficiency (CVID) after having suffered recurrent pulmonary infections for 10 years. A delay in the diagnosis of antibody deficiency is unfortunately not uncommon [1]. She received intravenous immunoglobulin (IVIG) replacement therapy with three weekly infusions of Vigam (BPL, Hertfordshire, UK) from 1995 onwards. During the period January 1997 to February 1998 she received batches of immunoglobulin that contained plasma from a donor who later developed variant Creutzfeldt–Jakob disease (vCJD). She received 8 × 5 g vials from batch VGD 049 and 4 × 2·5 g vials from VGD 050. The estimated ID50/g of these batches were 0·0000112 and 0·0000688, respectively. At age 72, she died of recurrence of adenocarinoma of the bowel.

Post-mortem analysis of tissues was performed by the National Creutzfeldt-Jakob Disease Surveillance Unit. She had been embalmed after death, by the introduction of formaldehyde into her femoral artery, but this process is not known to affect the detection of prion material in the body tissues. Western blotting of spleen and lymph nodes was negative for prion protein. There was no evidence of prion protein being present in the brain on histological, immunocytochemical or Western blot analysis. The time interval between treatment with the implicated batches and death from unrelated causes was 9 years, which is longer than the interval from transfusion to death in the reported cases of vCJD transmission by red cell components (5–8·5 years) [2]. Therefore, it seems reasonable to expect to find evidence of abnormal prions if transmission had occurred in this case.

Although the patient received IVIG from a batch containing plasma from a donor who developed vCJD, the patient did not develop vCJD clinically, and there was no evidence of prion protein deposition using histopathological and molecular techniques. There are no known cases of prion transmission by IVIG, in contrast to transfusions of red cell components where four cases have been reported to date [2]. The safety of pooled plasma products such as IVIG has been enhanced by adding to their manufacturing scheme multiple steps that reduce the potential for such transmission. Current IVIG manufacturing schemes are able to remove prion particles with up to a 5 log reduction [3,4] such that the risk of transmission of vCJD by IVIG may be low, even when a donation contains prion protein.

Although there have been no reports of vCJD transmission by IVIG, UK plasma has not been used for fractionation of pooled plasma products since 1997 as a (continuing) precautionary measure to avoid possible transmission. There are many indications for the use of IVIG [5], and worldwide demand exceeds supply. More stringent indications for its use are currently being drawn up and implemented in the UK (http://www.ivig.nhs.uk). Increasing difficulty in UK supply from the world market suggests that it may be appropriate to re-examine whether the ban on the use of UK plasma to make fractionated pooled plasma products should continue. We believe that this case highlights many of the issues surrounding the current debate.

Received: 24 October 2008,
revised 8 December 2008,
accepted 14 December 2008

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別紙様式第2-1

No. 4

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
				2008. 12. 18	該当なし	
一般的名称	新鮮凍結人血漿		研究報告の公表状況	Watson R. BMJ 2008 Nov; 337(7680)	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)				ベルギー	

研究報告の概要	○クラミジアは2006年に欧州でもっとも多く報告された感染症であることが新しいデータで示された。欧州疾病管理予防センター（ECDC）の調査によると、クラミジア症は2006年に欧州において225,000件を上回る症例が記録され、もっとも報告頻度の高い感染疾患であった。以下、ランブル鞭毛虫症（193,000症例）、カンピロバクター症（180,000症例）、サルモネラ症（168,000症例）と続き、ストックホルム研究所に定期的に報告される47感染症のうちの上位10位を占めた他の感染症は、結核、流行性耳下腺炎、淋病、C型肝炎、侵襲性肺炎球菌疾患、HIVであった。 結核症例数はEU加盟27カ国とアイスランド、ノルウェー、リヒテンシュタインで減少傾向を示したが、英国、オランダ、スイス、ノルウェー、スウェーデンなどの移民では50%以上増加した。毎年、欧州では約90,000名が結核と診断され7,800名が死亡する。主に男性と性的な接触をもつ男性のHIV感染は増加し、毎年約30,000名がHIV / AIDSの診断を受け1,800名が死亡する。2010年までに欧州での根絶を目指している麻疹は6,279症例を記録している。 季節性インフルエンザは、年間2,500万人～5,000万人が感染し約40,000人が死亡する。また、欧州では毎年400万人ほどが院内感染し37,000名が死に至る。 メチシリン耐性黄色ブドウ球菌（MRSA）に関する状況は2002年以降ベルギー、オーストリアとスロベニアでは改善されたが、それ以外の国は横ばいまたは増加した。抗生物質の不適切な使用が公衆衛生における重大な脅威を招くこと、抗生物質の有効性を保つことは自身の責任であるとしたキャンペーンが展開されている。
報告企業の意見	今後の対応
欧州における2006年の感染症の発生報告はクラミジアが最も多く、以下、ランブル鞭毛虫症、カンピロバクター症、サルモネラ症、結核、流行性耳下腺炎、淋病、C型肝炎、侵襲性肺炎球菌疾患、HIVの順であったとの報告である。	今後も情報の収集に努める。

使用上の注意記載状況・その他参考事項等
新鮮凍結血漿「日赤」 新鮮凍結血漿-LR「日赤」 血液を介するウイルス、細菌、原虫等の感染 vCJD等の伝播のリスク

(34)

Published 19 November 2008, doi:10.1136/bmj.a2622
Cite this as: BMJ 2008;337:a2622

News

Chlamydia was most often reported infection in Europe in 2006, new data show

Rory Watson

[1] Brussels

Just over 225 000 cases of chlamsydia were recorded in Europe in 2006, making it the most frequently reported infectious disease, the latest research by the European Centre for Disease Prevention and Control shows.s

The findings, which will be published in the Stockholm based centre's annual epidemiological report in a few weeks' time, also confirm that giardiasis was the second commonest disease, with 193 000 cases. This is considerably more than the 15 000 reported in 2005, but the increase is almost entirely due to the 170 000 cases that occurred in Romania.

Two other food and waterborne infections came in third and fourth place: campylobacteriosis (180 000 cases) and salmonellosis (168 000). Other infectious diseases to feature in the top 10 of the 47 that are routinely reported to the Stockholm agency were tuberculosis, mumps, gonorrhoea, hepatitis C, invasive pneumococcal disease, and HIV.

Andrea Ammon, head of the centre's surveillance unit, gave an early presentation of the report's contents at a meeting of the agency's management board in Paris last week.

She noted that although the number of cases of tuberculosis had tended to fall in the 27 European Union members and in Iceland, Norway, and Liechtenstein, increases of up to 50% or more were being found among immigrants in countries such as the United Kingdom, the Netherlands, Switzerland, Norway, and Sweden.

The report also confirms an increase in infections of HIV, mainly among men who have sex with men, and records 6279 cases of measles, a disease that Europe is committed to eradicate by 2010.

The centre says that some four million people in Europe are infected every year while being treated in hospitals or clinics, of whom 37 000 die as a result. Seasonal flu affects between 25 and 50 million people a year, killing around 40 000.

Each year some 90 000 diagnoses of tuberculosis are made, a disease that kills 7800 people, while HIV or AIDS is identified in about 30 000 people, 1800 of whom die from the disease.

Although the situation regarding meticillin resistant *Staphylococcus aureus* (MRSA) had improved in Belgium, Austria, and Slovenia since 2002, in all other countries the levels of resistance to MRSA had either remained the same or grown. Data presented by Dominique Monnet, programme coordinator for antimicrobial resistance, showed that a threefold gap exists between countries that prescribe antibiotics to outpatients the most and those that do so the least.

Drawing on the high profile information campaigns that have helped to reduce use of antibiotics in France and Belgium, the Stockholm centre has helped more than 30 countries throughout Europe to run antibiotic awareness events in recent weeks. The common messages at the different events are that inappropriate use of antibiotics poses a serious threat to public health and that ensuring that antibiotics remain effective is everyone's responsibility.

Cite this as: *BMJ* 2008;337:a2622

More information is at www.ecdc.europa.eu.

別紙様式第2-1

No. 5

医薬品 研究報告 調査報告書

識別番号・報告回数			報告日	第一報入手日	新医薬品等の区分	総合機構処理欄
				2008. 12. 19	該当なし	
一般的名称	新鮮凍結人血漿		研究報告の公表状況	FDA, CBER. Available from: http://www.fda.gov/cber/blood/fatal07.pdf.	公表国 米国	
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)					

研究報告の概要

○FDAに報告された供血後及び輸血後の死亡例 2007年度概要

2005年度から2007年度にかけて米国食品医薬品局(FDA)に報告された供血後及び輸血後の死亡例の概要である。

2007年度に、FDAは受血者76件、供血者17件の死亡報告を受領した。受血者死亡例の内訳は、52件が輸血に関連したもの、11件が死亡原因として輸血を排除できないもの、13件が輸血と関連しないものであった。

過去3年間の合計は177例で、内訳はTRALIが98件(55%)で最も高く、微生物感染は21件(12%)であった。微生物感染の内5件(24%)をバベシア症が占め、ついで*Staphylococcus aureus*が4件(19%)となった。

アフェレーシス血小板に関連した致死性の微生物感染報告は、2005年度から2006年度にかけて減少が見られ、2007年度も低いままであった。

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」

血液を介するウイルス、細菌、原虫等の感染
vCJD等の伝播のリスク

報告企業の意見	今後の対応
2005年度から2007年度にかけて米国食品医薬品局に報告された供血後及び輸血後の死亡例の概要である。	日本赤十字社では、薬事法及び関連法令に従い輸血副作用・感染症情報を収集し、医薬品医療機器総合機構を通じて国に報告している。今後も引き続き輸血副作用・感染症に関する情報の収集に努める。

Fatalities Reported to FDA Following Blood Collection and Transfusion

Annual Summary for Fiscal Year 2007

I. Background

As previously mentioned in the annual summary of fatalities reported to the FDA in Fiscal Years (FY) 2005 and FY2006, the blood supply is safer today than at any time in history. Due to advances in donor screening, improved viral marker tests, automated data systems, and changes in transfusion medicine practices, the risks associated with blood transfusion continue to decrease. Overall, the number of transfusion related fatalities reported to the FDA remains small in comparison to the total number of transfusions. In 2006 there were approximately 30 million components transfused.[1] During the proximate period of FY2006, there were 73 reported transfusion related and potentially transfusion related fatalities, with a decrease to 63 in FY2007.

CBER is distributing this summary of transfusion fatality reports received by the FDA to make public the data received in FY2007, to provide the combined data received over the last three fiscal years, and to compare the FY2007 reports to the fatality reports received in FY2006 and FY2005. We also include information on the infrequent reports of post-donation fatalities. Throughout this report we note changes over time, but the reader should interpret these changes cautiously, given the small numbers of reports and inherent variations in reporting accuracy. The significance of shifts in numbers derived from small populations may appear to be greater than they really are.

Refer to Sections 606.170(b) and 640.73 of Title 21, Code of Federal Regulations (21 CFR 606.170(b) and 21 CFR 640.73), for fatality reporting requirements. For information regarding the notification process, see our web page, Notification Process for Transfusion Related Fatalities and Donation Related Deaths, http://www.fda.gov/cber/transfusion.htm. For further information, see our *Guidance for Industry: Notifying FDA of Fatalities Related to Blood Collection or Transfusion*, September 2003.[2]

1 Whitaker BI, Green J, et al. The 2007 Nationwide Blood Collection and Utilization Survey Report. Washington (DC): Department of Health and Human Services; 2008.

2 Guidance for Industry: Notifying FDA of Fatalities Related to Blood Collection or Transfusion, September, 2003. http://www.fda.gov/cber/gdlns/bldfatal.htm.

If you have questions concerning this summary, you may contact us using any of the three following options.

1. Email us at fatalities2@fda.hhs.gov,
2. Call us at 301-827-6220, or
3. Write us at:
   FDA/Center for Biologics Evaluation and Research
   Office of Compliance and Biologics Quality
   Division of Inspections and Surveillance (HFM-650)
   1401 Rockville Pike, Suite 200 North
   Rockville, Maryland 20852-1448

II. Results

During FY2007 (October 1, 2006, through September 30, 2007), we received a total of 93 fatality reports. Of these reports, 76 were transfusion recipient fatalities and 17 were post-donation fatalities.

Of the 76 transfusion recipient fatality reports, we concluded:
a) 52 of the fatalities were transfusion-related,
b) in 11 cases we were unable to rule out transfusion as the cause of the fatality,
c) 13 of the fatalities were unrelated to the transfusion.

We summarize the results of our review in the following sections. Sections A through D of this document present the transfusion-related fatalities. Sections E and F and Table 4 present the fatality reports which were unrelated to the transfusion, or in which we could not rule out the transfusion as the cause of death. Section G presents the post-donation fatality reports.

A. Overall Comparison of Transfusion-Related Fatalities Reported in FY2005, FY2006, and FY2007

In combined FY2005, FY2006, and FY2007, Transfusion Related Acute Lung Injury (TRALI) caused the highest number of reported fatalities (55%), followed by hemolytic transfusion reactions (22%) due to non-ABO (15%) and ABO (7%) incompatibilities. Complications of

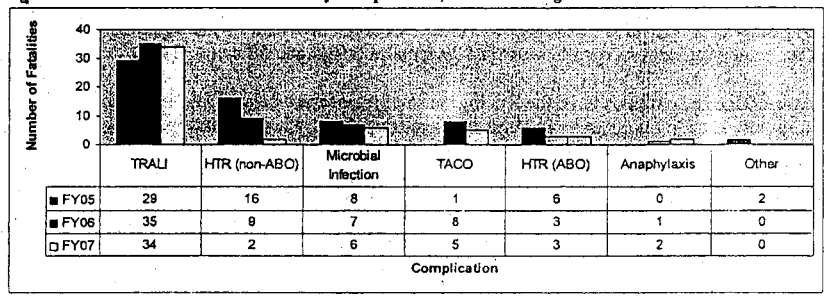
microbial infection, Transfusion Associated Circulatory Overload (TACO), and anaphylactic reactions each accounted for a smaller number of reported fatalities (Table 1and Figure 1).

Table 1: Transfusion-Related Fatalities by Complication, FY2005 through FY2007

	FY05		FY06		FY07		Total (FY05+06+07)	
Complication	No.	%	No.	%	No.	%	No.	%
TRALI	29	47%	35	56%	34*	65%	98	55%
HTR (non-ABO)	16	26%	9	14%	2	4%	27	15%
Microbial Infection	8	13%	7	11%	6	12%	21	12%
TACO	1	2%	8	13%	5	10%	14	8%
HTR (ABO)	6	10%	3	5%	3	6%	12	7%
Anaphylaxis	0	0%	1	2%	2	4%	3	2%
Other	2**	3%	0	0%	0	0%	2	1%
Totals	62	100%	63	100%	52	100%	177	100%

*In FY2007, our review committee began using the Canadian Consensus Conference criteria[3,4] for evaluating TRALI cases – this number includes both "TRALI" and "possible TRALI" cases
**Other: Includes one case of Graft vs. Host Disease (GVHD) and one therapeutic plasma exchange (TPE) error (use of a treatment column contraindicated due to patient's medical history)

Figure 1: Transfusion-Related Fatalities by Complication, FY2005 through FY2007



B. Transfusion Related Acute Lung Injury (TRALI)

In FY2007, as in the previous two fiscal years, TRALI continued to be the leading cause of transfusion related fatalities reported to CBER, representing 65% of confirmed transfusion related fatalities. Over the last three fiscal years, TRALI represented 55% of confirmed transfusion related fatalities. While the number of TRALI fatalities associated with receipt of Fresh Frozen Plasma (FFP) decreased from 22 (63% of TRALI cases) in FY2006 to 12 (35% of

[3] Goldman M, Webert KE, Arnold DM. et al. Proceedings of a consensus conference: towards an understanding of TRALI. Transfus Med Rev 2005;19:2-31.
[4] Kleinman S, Caulfield T, Chan P, et al. Toward an understanding of transfusion-related acute lung injury: statement of a consensus panel. Transfusion 2004;44:1774-1789

TRALI cases) in FY2007 (Figure 2), the number was comparable to that reported in FY2005 (13 cases). For the same three years there was an increase in reports of TRALI fatalities from Red Blood Cells (RBC) with 5 cases reported in each of FY2005 and FY2006 compared with 12 cases reported in FY2007.

When compared to the proportions of all transfused products, plasma products continue to be associated with a disproportionate share of TRALI cases. In Calendar Year 2006, for example, transfused plasma products accounted for approximately 13% of all transfused components, apheresis platelets (using platelet concentrate equivalent units) – approximately 30%, and red blood cell-containing products – approximately 49%.[5] In comparison, for the combined fiscal years 2005-2007, FFP and other plasma accounted for 52% (51/98) of reported TRALI fatalities, apheresis platelets accounted for 7% (7/98), and RBC's accounted for 22% (22/98).

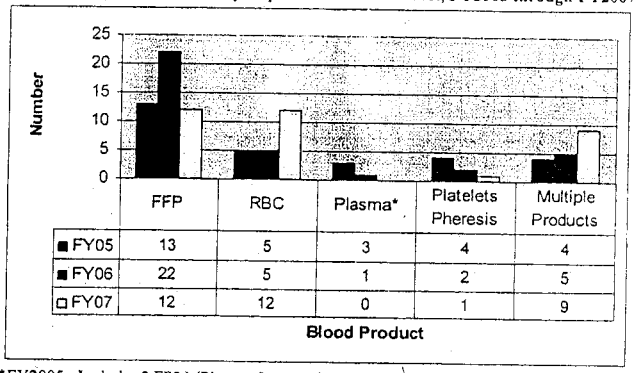
In FY2007, there were 34 TRALI cases temporally associated with products from 162 donors. Of these donors, 104 (64%) were tested for white blood cell (WBC) antibodies (Table 2). Antibody tests were negative in 41% of those tested. Of those tested, Human Leukocyte Antibodies (HLA) were present in 43% of donors. Human Neutrophil Antibodies (HNA) were present in 22% of donors, but most of these reactions (12/17) were weak and non-specific. Many donors had multiple antibodies. Reporters who included patient testing data were able to match donor antibodies with recipient cognate antigens in 7 of the 34 cases, implicating 11 donors (In 2 of these cases, there were recipient matches with 3 donors).

The gender of 25 (15%) of the donors was unknown or not provided by the reporting facilities. Of the remaining donors, reports identified 79 females (49%) and 58 males (36%).

Because TRALI continues to be the leading cause of transfusion-related fatalities, the transfusion community is taking voluntary measures to reduce this risk. Data show that the largest percentage of fatal TRALI cases are associated with female donors with white blood cell antibodies, and recent literature describes efforts to selectively use plasma from male donors for transfusion.[6,7,8] In November, 2006, the American Association of Blood Banks (AABB) issued an Association Bulletin, which included a recommendation that blood collection and transfusion facilities begin implementation of TRALI risk reduction measures for all high plasma-volume components. The measures include interventions to minimize the preparation of these components from donors known to have white blood cell antibodies or who are at increased risk for developing these antibodies.[9]

[5] Whittaker BI, op.cit. Tables 4-1 and 4-2.
[6] Curtis, BR, Mcfarland JG. Mechanisms of transfusion-related acute lung injury (TRALI): anti-leukocyte antibodies. Crit Care Med 2006;34(5 Suppl):S118-S123.
[7] Eder AF, Herron R, Strupp A, et al. Transfusion-related lung injury surveillance (2003-2005) and the potential impact of the selective use of plasma from male donors in the American Red Cross. Transfusion 2007;47:599-607.
[8] Chapman CE, Williamson LM, Cohen H, et al. The impact of using male donor plasma on hemovigilance reports of transfusion-related acute lung injury (TRALI) in the UK (abstract). Vox Sang 2006;91(Suppl 3):227.
[9] Transfusion-related acute lung injury. AABB Association Bulletin. Bethesda: American Association of Blood Banks;2006 Nov 3.

Figure 2: Reports of TRALI by Implicated Blood Product, FY2005 through FY2007



*FY2005: Includes 2 FP24 (Plasma frozen within 24 hours after collection) and 1 Liquid Plasma
FY2006: Includes 1 FP24

Table 2: Donor Antibodies Identified in Association with TRALI, FY2007

FY07 Donor Leukocyte Antibodies	No.	%
HLA Class I	18	17%
HLA Class II	6	6%
HLA Class I and II	15	14%
HNA	17	16%
HLA and HNA	6	6%
Negative	42	41%
Total Donors Tested	104	100%

This table does not include the 59 donors that were not tested for WBC antibodies

C. Hemolytic Transfusion Reactions

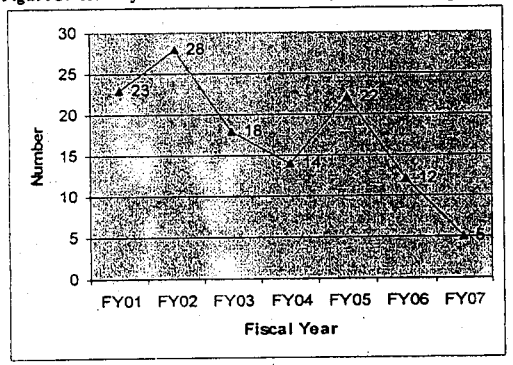
In FY2007, there was a continued decline in the number of reported fatal hemolytic transfusion reactions, with a total of five, as compared to 12 in FY2006, and 22 in FY2005. The recent decrease is due to a decline in reports of non-ABO hemolytic reactions, with reports of 16 fatalities in FY2005, 9 in FY2006 and 2 in FY2007 (Figure 1 and Table 3). We have seen an overall decrease in the number of reported fatal hemolytic transfusion reactions since FY2001 (Figure 3).

Table 3: Hemolytic Transfusion Reactions by Implicated Antibody, FY2005 through FY2007

	FY05		FY06		FY07		Total (FY05+06+07)	
Antibody	No.	%	No.	%	No.	%	No.	%
ABO	6	27%	3	25%	3	60%	12	31%
Multiple Antibodies*	6	27%	4	33%	1	20%	11	28%
Other**	3	14%	0	0%	0	0%	3	8%
Jk^b	3	14%	0	0%	0	0%	3	8%
Jk^a	1	5%	1	8%	1	20%	3	8%
K	1	5%	1	8%	0	0%	2	5%
Fy^a	0	0%	1	8%	0	0%	1	3%
Fy^b	0	0%	1	8%	0	0%	1	3%
E	1	5%	0	0%	0	0%	1	3%
I	1	5%	0	0%	0	0%	1	3%
Js^a	0	0%	1	8%	0	0%	1	3%
Totals	22	100%	12	100%	5	100%	39	100%

*FY2005 antibody combinations included E+c, Fy^a+K, Fy^a+Jk^b, E+I+A_1, possible C+E+K, Wr^a+warm autoantibody.
*FY2006 antibody combinations included E+c, S+K, Jk^b+cold agglutinin, unidentified auto- and alloantibodies.
*FY2007: anti-M+C
**Includes one report of non-immune hemolysis, one report of an unidentified antibody to a low incidence antigen, and one report of Cold Agglutinin Syndrome due to *Mycoplasma pneumonia* or Lymphoma.

Figure 3: Hemolytic Transfusion Reactions, FY2001 through FY2007



In FY2007, there were three reports of fatal hemolytic transfusion reactions due to ABO-incompatible blood transfusions:

- 1 case: recipient identification error at the time of transfusion
- 1 case: blood bank clerical error (incorrect sample used for testing)
- 1 case: initial recipient ABO/Rh typing results switched with another patient; ABO incompatible FFP issued prior to completion of required second typing

D. Microbial Infection

In FY2007, there were 6 reported fatalities attributed to microbial infection compared with 7 reported in FY2006 and 8 reported in FY2005. Three different bacteria were implicated in three fatalities, and three other fatalities resulted from Babesia transmission following Red Blood Cell transfusions from donors who subsequently tested positive for *Babesia microti*. The babesiosis cases accounted for 50% (3/6) of the microbial infections associated with transfusion fatalities in FY2007. Babesia accounted for 24% (5/21) of reported cases over the last three fiscal years, followed by Staphylococcus aureus, which accounted for 19% (4/21) (Table 4).

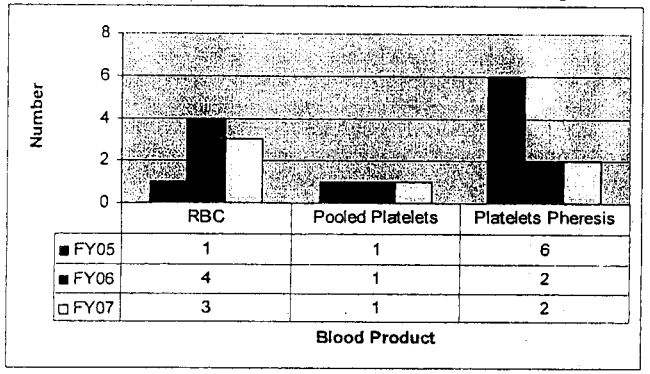
There was one strict anaerobe, *Eubacterium limosum*, implicated in a fatal bacterial infection during the 3-year reporting period; this fatality occurred in FY2005. The remaining bacteria are facultative anaerobes.

In FY2007, the decrease in reports of fatal microbial infections associated with apheresis platelets seen between FY2005 and FY2006 persisted (Figure 4). This finding is consistent with an overall decrease in the number of bacterial infections associated with apheresis platelets since FY2001 (Figure 5).

Table 4: Microbial Infection by Implicated Organism, FY2005 through FY2007

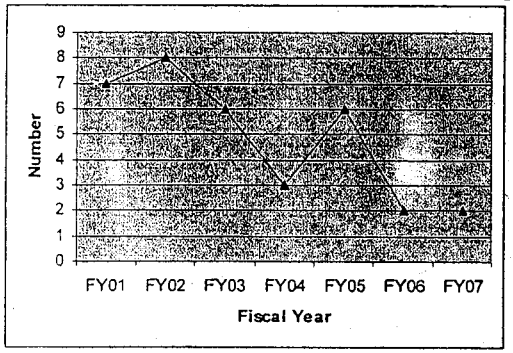
	FY05		FY06		FY07		Total (FY05+06+07)	
Organism	No.	%	No.	%	No.	%	No.	%
Babesia microti	0	0%	2	29%	3	50%	5	24%
Staphylococcus aureus	3	37%	0	0%	1	17%	4	19%
Escherichia coli	0	0%	3	43%	0	0%	3	14%
Serratia marcescens	2	24%	0	0%	0	0%	2	10%
Staphylococcus lugdunensis	1	13%	0	0%	0	0%	1	5%
Staphylococcus epidermidis	1	13%	0	0%	0	0%	1	5%
Eubacterium limosum	1	13%	0	0%	0	0%	1	5%
Morganella morganii	0	0%	1	14%	0	0%	1	5%
Yersinia enterocolitica	0	0%	1	14%	0	0%	1	5%
Streptococcus dysgalactiae	0	0%	0	0%	1	17%	1	5%
Klebsiella oxytoca	0	0%	0	0%	1	17%	1	5%
Total	8	100%	7	100%	6	100%	21	100%

Figure 4: Microbial Infection by Implicated Blood Product, FY2005 through FY2007



Red Blood Cells microorganisms: *S. marcescens (1), E. coli (1), Y. enterocolitica (1), B. microti (5)*
Pooled Platelets microorganisms: *S. aureus (1), E. coli (1), Streptococcus dysgalactiae (1)*
Platelets Pheresis microorganisms: *S. aureus (3), S. marcescens (1), S. lugdunensis (1), S. epidermidis (1), E. limosum (1), E. coli (1), M. morganii (1), K. oxytoca (1)*

Figure 5: Bacterial Infection by Apheresis Platelets, FY2001 through FY2007



E. Transfusion Not Ruled Out as Cause of Fatality

In these reported fatalities, the reporting facilities were unable to identify a specific complication of transfusion as the cause of death. Often, these patients had multiple co-morbidities, and after review of the investigation documentation, our medical reviewers could neither confirm nor rule out the transfusion as the cause of the fatality (Table 5). We did not include these reported fatalities in the analysis in Sections II.A through II.D (transfusion-related fatalities), above.

Combining the transfusion related fatalities with those that our medical officers could not rule out, there was a decrease in total reported fatalities from 73 in FY2006 to 63 in FY2007.

F. Not Transfusion Related

After reviewing the initial fatality reports and the investigation documentation, we categorized a number of reported fatalities as "Not Transfusion Related." Our medical reviewers concluded that, while there was a temporal relationship between transfusion and subsequent death of the recipient, there was no evidence to support a causal relationship (Table 5). Thus, we did not include these reported fatalities in the analysis in Sections II.A through II.D (transfusion-related fatalities), above.

Table 5: Fatalities Not Related to Transfusion or Transfusion Not Ruled Out, FY2005 through FY2007

	FY05	FY06	FY07
Not Transfusion Related	21	8	13
Not Ruled Out	14	10	11
Totals	35	18	24

G. Post-Donation Fatalities

There was a small increase in the number of fatalities following Source Plasma donation, and two fatalities following donation of Apheresis Platelets (Table 6). In two cases (both Source Plasma donors), our medical reviewers determined that clear medical evidence supported a cause of death that was not donation related. For the remaining 13 of the 15 FY2007 fatalities following Source Plasma and Apheresis Platelet donations, our medical reviewers concluded that, while there was a temporal link between the donations and the fatalities, there was no evidence to support a causal relationship between the Source Plasma or Apheresis Platelet donations and subsequent death of the donors. This was also the case for the 12 fatalities following Source Plasma donation in FY2005 and FY2006.

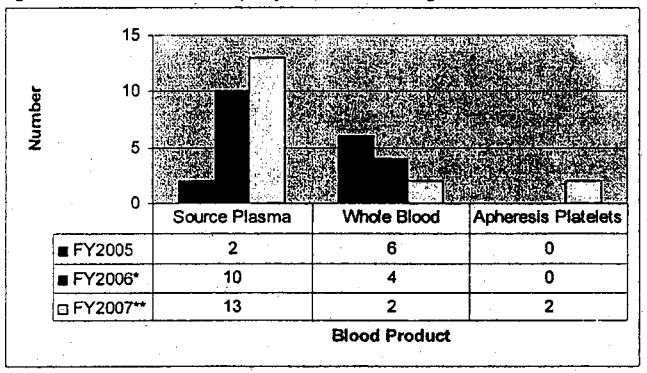
In FY2007, we received reports of two fatalities following Whole Blood donation, both autologous, collected by manual methods. In both cases, our medical reviewers found no evidence to support a causal relationship between the donation and subsequent death of the donor. For eight of the nine Whole Blood donations (includes two autologous donations) reported in FY2005 and FY2006, our medical reviewers found no evidence to support a causal relationship between the donation and subsequent death of the donor. In one FY2006 case, an autologous donation, our medical reviewers could neither confirm nor rule out the donation as contributing to the donor's death.

Table 6: Post-Donation Fatality Reports by Donated Product, FY2005 through FY2007

Donated Product	FY05	FY06	FY07
Source Plasma	2	10	13
Whole Blood	6	4*	2**
Apheresis Platelets	0	0	2
Total	8	14	17

*Includes 2 autologous donations
**Autologous donations

Figure 6: Post-Donation Fatality Reports, FY2005 through FY2007



*Includes 2 autologous Whole Blood donations
**Both Whole Blood donations in FY07 were autologous

別紙様式第2-1

No. 6

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
				2008. 11. 20	該当なし		
一般的名称	新鮮凍結人血漿		研究報告の公表状況	Gubernot D, Lucey C, Lee K, Conley G, Holness L, Wise R. AABB Annual Meeting and TXPO 2008; 2008 Oct 4-7; Montreal.	公表国	米国	使用上の注意記載状況・その他参考事項等
販売名(企業名)	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[日赤] 新鮮凍結血漿-LR[日赤]

研究報告の概要

○輸血を介したパベシア症の伝播:FDAに届けられた最近の死亡報告

背景:パベシア症は輸血を介した伝播リスクが知られているが、認可されたスクリーニング法は存在しない。本試験は、FDAに報告されたパベシア関連輸血事象の重症度と特徴について、最近の輸血関連パベシア症死亡報告と生物学的製品逸脱報告サマリー(BPDRs)に焦点を当て検討した。

方法:過去10年間にFDAに報告された3つのFDA調査システム(採血および輸血死亡報告、MedWatchプログラム、BPDRs)のデータを収集した。

結果:輸血感染パベシア症死亡報告は1998年の1例以降しばらく無かったが、2006年1月～10月にはFDAに5例が報告された。受血者は関連血液製剤の輸血から4～7週間後に発症し、全員が*Babesia microti*に感染していた。過去10年間のパベシア症関連のBPDRsは68件であり、近年この報告が増加傾向にあることは、当該寄生虫による輸血関連リスクが増加していることを示している。

結論:最近の死亡報告は、増加中のBPDRsと合わせて、稀な輸血後合併症であるパベシア症のリスク増大を明らかにした。発熱を呈した受血者にはパベシア症の可能性があることを医師が認識することにより、効果的治療のための迅速な診断を容易にし、また、残存する血液製剤を差止める検査の実施が促進されると考える。パベシア症供血者および輸血関連事象の報告は、FDAによるリスク範囲の評価、公衆衛生上の感染制御対策の一助となる。

報告企業の意見	今後の対応
FDAに報告されたパベシア関連輸血事象の重症度と特徴について、最近の輸血関連パベシア症死亡報告と生物学的製品逸脱報告サマリーに焦点を当て検討した結果、近年、当該寄生虫による輸血関連リスクが増加していることを示しているとの報告である。	今後も引き続き、新興・再興感染症の発生状況等に関する情報の収集に努める。

(3b)

SP246

Quantitative Real-time PCR Assay for *Trypanosoma cruzi*

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Background: *Trypanosoma cruzi* infects about 18 million people, and results in 50,000 deaths from Chagas disease annually, primarily in Latin America. Latin American blood donors in the US may harbor chronic *T. cruzi* infection and be potential reservoir of *T. cruzi* transmission by blood transfusion. US blood centers began donor screening for *T. cruzi* antibody (Ab) in early 2007 and have identified hundreds of seropos blood donors. Our objective was to develop a sensitive assay for *T. cruzi* parasite detection and quantitation in whole blood (WB) samples from seropos donors. The assay is also needed for studies of *T. cruzi* transfusion-transmission and disease pathogenesis. **Methods:** Trypomastigotes of *T. cruzi*, grown in culture, were harvested, counted, and spiked into fresh WB to create samples containing 8, 4, 2, and 1 parasite/20 mL WB. Lysis of parasites was performed by adding 20 mL of Guanidium-EDTA lysis buffer (6M Guanidine HCl with 0.2M EDTA, pH8.0) to 20 mL WB and vortexing. The lysed WB was heated at 100C for 15 mins to disentangle minicircle kinetoplast DNA present at ~10,000 copies/parasite. Total DNA was prepared from 0.4 mL of the lysate by precipitating hemoglobin and inhibitors. Parasitic DNA was captured by *T. cruzi* specific oligonucleotide probes bound to magnetic beads. After being eluted from the beads, parasite DNA was amplified by real-time (RT)-PCR with SyBr green dye & an optimized buffer system using a *T. cruzi* kinetoplast DNA specific primer pair (Tc-121/Tc-S36). **Results:** Table summarizes RT-PCR results for 5 replicate amplifications of the spiked dilution series. A single parasite in 20 mL WB gave strong signal (~10 cycles below 45-cycle cutoff) & good precision quantitation of up to 8 parasites. We tested 27 coded specimens from *T. cruzi* Ab-reactive donors: 2/7 RIPA(+) and 0/20 RIPA(–) donors tested PCR(+); the 2 pos donors had ~1 parasite/20 mL WB. **Conclusion:** We can detect single *T. cruzi* parasites in 20 mL WB with this sensitive quantitative RT-PCR assay. Additional *T. cruzi* seropos donor blood samples from the US, Argentina, Honduras & Brazil are being collected for analysis.

	# of *T. cruzi* Spiked into 20 mL Whole Blood				
n = 5	8	4	2	1	0
Mean Cp (±STD)	31.4 (±0.5)	32.64 (±0.1)	33.48 (±.01)	15.18 (±~0.0)	>45

A one unit change in Cp in a real-time PCR assay is expected to equate to an ~ doubling of parasite load. Our assay performs as expected in the range of 1-8 parasites.

Disclosure of Conflict of Interest

Tzong-Hae Lee, Ester Sabino, Lani Montalvo, Li Wen, Daniel Chafets, Brian Custer, Michael P. Busch, for the Retrovirus Epidemiology Donor Studies-II (REDS-II): Nothing to Disclose

SP247

Screening for *Trypanosoma cruzi* in the Blood Donor Setting

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Background: Our blood donor center recently began testing for antibodies to the agent that causes Chagas' Disease (*Trypanosoma cruzi*). We reviewed incidence among our current blood donor population and all look-back cases to determine if there were any reports of transfusion-transmitted *Trypanosoma cruzi*. **Methods:** At our center all allogeneic and autologous donations were tested for antibodies to *T. cruzi* using a US Food and Drug Administration licensed enzyme immunoassay (EIA) methodology. Those donations that were repeat reactive (RR) on EIA were sent for an unlicensed confirmatory radioimmunoprecipitation assay (RIPA). In accordance with AABB Association Bulletin 06-08 donors RR on EIA were indefinitely deferred and notified of results. Look-back was performed on those donors who tested RIPA positive and included all electronic donor records available. **Results:** From 7/30/07-3/15/08 222,059 donations (212,505 whole blood, 7,520 autologous, 2,034 directed and of which 51,298 were first-time donors) were tested by EIA for anti-*T. cruzi*. 16/222,059 (0.007%) donations were EIA RR donations. Confirmatory RIPA results were as follows: 7/16 (43.75%) or 7/222,059 (0.003%) were positive and 9/16 (56.25%) were negative. 2/7 (28.6%) or 2/51,298 (0.004%) RIPA positive results were from first-time donors. Look-back was performed on the 5 RIPA positive repeat donors and involved 75 transfusable blood components (70 were transfused, 2 discarded and 3 no information was provided). There were no reports of recipients of the 70 transfused blood components testing reactive for antibodies to *T. cruzi*. **Conclusions:** At our blood center, the introduction of testing for *T. cruzi* prevented transfusion of a small number of units that confirmed positive for the presence of antibodies. Look-back revealed no reports of transfusion-transmission of *T. cruzi* from previously donated untested units.

Disclosure of Conflict of Interest

Richard Gammon, Michael Pratt: Nothing to Disclose

TTID 2: Tideborne Disease, CJD

SP248

A Fatal Case of Transfusion-Transmitted Babesiosis in the State of Delaware

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Background: Babesiosis is an emerging zoonotic disease caused by intraerythrocytic protozoa. Although the disease is usually transmitted by tick bite, there has been an increase in the number of transfusion-transmitted cases reported. This report describes a fatal case of transfusion-transmitted babesiosis in Delaware. **Case Report:** The patient was a 43-year-old Caucasian woman with history of transfusion-dependent Diamond-Blackfan Syndrome, hepatitis C, pulmonary hypertension and splenectomy. She had been receiving two units of RBCs every 2 weeks. She presented on 1/9/08 with fever, chills, cough and fatigue, and was treated with antibiotics initially for presumptive pneumonia. Examination of the peripheral blood smears revealed numerous intraerythrocytic ring forms, consistent with Babesia. The diagnosis of babesiosis was confirmed by positive polymerase chain reaction (PCR) for B. microti DNA and high titer of antibody to B. microti (1:2048). Despite aggressive therapy including Clindamycin and Quinine, the patient's condition rapidly deteriorated with multi-system organ failure and she expired 3 days after admission. The patient resided in Delaware and had no history of tick bites or recent travel history outside Delaware. Thirteen implicated donors were subsequently tested for B. microti. All tested donors were negative by PCR for B. microti. However, one of them had a significantly elevated B. microti antibody titer (1:1024). This donor resides in New Jersey and had recently traveled to Rhode Island. The donor has no known history of tick bites or flu-like symptoms within the past 2 years. The donor has not been diagnosed with Babesiosis, Lyme's disease or Ehrlichiosis, and has never received a blood transfusion. The implicated unit was donated on 8/8/07, frozen, and transfused as a deglycerolized unit on 11/27/07, 6 weeks prior to development of the patient's symptoms. **Conclusion:** This case emphasizes the need to review peripheral blood smears in febrile, immunocompromised patients who have been recently transfused. Prompt recognition and treatment are important, as Babesia infections can be severe or fatal in splenectomized and/or immunocompromised patients. It also illustrates the need for better strategies, including more sensitive, specific and rapid screening tests, to prevent transfusion-transmitted babesiosis.

Disclosure of Conflict of Interest

Yong Zhao, Ken Love, Scott Hall, Frank Beardell: Nothing to Disclose

SP249

Babesiosis Transmission through Blood Transfusion: Recent Fatality Reports Received by FDA

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Background: Babesiosis is a known transfusion-transmitted disease risk, with no licensed donor screening assay. There are estimates that 70 transfusion-transmitted cases have occurred from 1979 through 2007. This research evaluated the magnitude and characteristics of Babesia-related transfusion events reported to the Food and Drug Administration (FDA) with focus on the recent transfusion-related babesiosis fatality reports and a summary of Biological Product Deviation Reports (BPDRs) submitted to the FDA. **Methods:** Data were collected by querying three FDA surveillance systems for reports received within the past decade: Blood Collection and Transfusion Fatality Reporting, the MedWatch Program, and BPDRs. **Results:** Between January and October 2006, the FDA received five transfusion-related babesiosis fatality reports after only one prior report in 1998. Recipients presented with symptoms 4 to 7 weeks after transfusion of implicated blood units, and all were infected with Babesia microti. No MedWatch report was received; however 68 Babesia-related BPDRs over the past decade, with increasing numbers in more recent years, suggest a rising risk for transfusion-transmission from this parasite. **Conclusions:** The recent fatality reports, along with growing numbers of BPDRs, underscore babesiosis as a rare post-transfusion complication whose risk may be increasing. Enhanced clinician awareness of the possibility of babesiosis in febrile transfusion recipients may facilitate prompt diagnosis with more effective treatment and timely investigations to interdict extant infected units. Reporting of babesiosis donor and transfusion-related events assists the FDA in assessing the scope of the risk and developing appropriate public health control measures. Disclaimer: The findings and conclusions in this abstract have not been formally disseminated by the Food and Drug Administration and should not be construed to represent any Agency determination or policy.

Disclosure of Conflict of Interest

Diane Gubernot, Charles Lucey, Karen Lee, Gilliam Conley, Leslie Holness, Robert Wise: Nothing to Disclose

SP250

Evaluation of Candidate Reentry Proposals for *Babesia microti* Infection

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Background: B. microti (Bm) is a tick-borne rbc parasite which can be transmitted by transfusion from chronically infected donors. Implication in transfusion babesiosis (TB) or clinical babesiosis (CB) requires permanent donor deferral. As part of a multi-year longitudinal research study in New England, Bm seropositive blood donors are deferred despite apparent clearance of infection in many cases. We evaluated several candidate donor reentry proposals (Schemes) that also may be applicable to donors with CB or implicated in TB. **Methods:** Consenting blood donors were screened by IFA for Bm (positive ≥1:64) using retention tubes (index sample). Consenting positive donors agreed to provide subsequent samples at 1-2 month intervals which were screened by IFA and nested- or RT-PCR. 18 donors were released from study before 1 year after 3 consecutive negative bleeds. 45 donors dropped out and could not be evaluated. Study data were used to evaluate 4 potential reentry Schemes based on the initial PCR result (<12 week after the initial IFA) and on the first IFA and PCR result ≥11 months following the index sample (Table 1). Reentry failure was defined as a PCR positive samples following successful reentry. **Results:** 76/139 donors completed 1 year or more of follow-up and were eligible for assessment using the four candidate reentry Schemes (Table 2). All 43 eligible donors with IFA titers ≤1:128 after the index sample could be reentered. Only 21/33 (64%) donors with 1 or more IFA titers >1:128 after the index sample could be reentered. Requiring all IFA titers to be ≤1:128 would eliminate only 1/3 Scheme failures, but would require multiple donor samples. Requiring 2 rather than 1 year wait after the seropositive screen would eliminate the observed Scheme failures in all cases. However, this could not be fully assessed because of limited follow-up. **Conclusion:** Reentry for Bm is feasible using approaches similar to other TTD markers. Evaluated Schemes could reenter a significant portion of donors; however, there was a small, but unacceptable failure rate. In addition, 18 donors released from the study before a year could also be considered for reentry, but there was no follow-up to assess this approach. Sampling beyond 1 year may be required to develop an acceptable reentry Scheme. Such a Scheme could be useful for donor management if Bm screening is implemented, and could allow reentry of donors implicated in TB or recovered CB.

TABLE 1. Reentry schemes

#	Initial IFA	Initial PCR	IFA 1 Year	PCR 1 Year	Other PCR
1a	≥1:64	Neg	≤1:128	Neg	All Neg
1b	≥1:64	Neg	≤1:128	Neg	Any
2a	≥1:64	Pos or NA	≤1:128	Neg	All Neg
2b	≥1:64	Pos or NA	≤1:128	Neg	Any

TABLE 2. Evaluation of reentry schemes

Reentry scheme	1a	1b	2a	2b
Eligible initially	116	116	139	139
Followed 1 year	55	55	76	76
Reentered	42	47	55	64
% reentered	76%	85%	72%	84%
Scheme failures*	2	3	2	3

* PCR positive samples following successful reentry

Disclosure of Conflict of Interest

Ritchard Cable, Stephanie Johnson, Laura Tonnetti: Nothing to Disclose
David Leiby: Not Specified

SP251

Seasonal and Geographic Distribution of *Babesia microti* Seroprevalence in Connecticut Blood Donors: 2006 and 2007

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Background: *Babesia microti* is an intraerythrocytic parasite, transmitted by *Ixodes* ticks, that is found throughout the northeastern United States. *B. microti* is also transmitted by blood transfusion, with over 70 cases reported to date. Individuals exposed to the parasite may develop babesiosis, a potentially life threatening illness. Those at greatest risk for developing serious disease include asplenic, elderly and immunocompromised individuals. Our blood center has been studying the presence of antibodies to *B. microti* in Connecticut blood donors since 1999. The purpose of this analysis is to provide data, and highlight the need, for the development of methods for screening the blood supply to improve blood safety. **Methods:** Consenting blood donors are tested at select blood drives. A donor is considered seropositive when they test positive for *B. microti* antibodies by IFA (≥1:64). Beginning in 2006 testing was conducted year round and included blood drives in all eight counties of Connecticut. **Results:** Seropositive individuals were identified in every county (Table 1), although the two southeastern counties (Middlesex and New London) each had significantly higher seroprevalence rates when compared to the remaining six counties ($p < 0.05$ for both). Seropositive individuals were identified in every month and seroprevalence varied month to month but there was no apparent seasonal pattern. **Conclusions:** Seroprevalence of *B. microti* in Connecticut varies significantly by county, but every county had substantial seroprevalence, 0.4% or greater seropositive rate (40/10,000 donors). Seropositive donors were identified in every month of the year. Based on these results, using seasonal or geographic exclusion criteria to interdict *Babesia* from the blood supply would be an ineffective approach. These data support the need for developing efficient methods for screening the blood supply for *Babesia*, and thereby improving blood safety.

TABLE 1. 2006 & 2007

County	# Tested	# Positive	Seroprevalence per 10,000 Donors
Fairfield	1631	10	61
Hartford	2609	17	65
Litchfield	375	2	53
Middlesex	654	10	153
New Haven	1521	10	66
New London	1062	19	179
Tolland	418	3	72
Windham	252	1	40

Disclosure of Conflict of Interest

Stephanie Johnson, Ritchard Cable, Eric Van Tassell, Laura Tonnetti: Nothing to Disclose
David Leiby: Not Specified

SP252

Transfusion Transmitted Babesiosis in an ITP Patient: A Case Report

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Our case is a 79 years old male who presented to Danbury Hospital Emergency Department (ED) complaining of fever and chills that started a few hours earlier. The patient was discharged 2 weeks prior following a Clostridium difficile (C. difficile) infection. On physical examination the patient