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研究報告の概要

チクングニアウイルス(CHIKV)流行は、以前アフリカ、インド洋諸島、南アジアと東南アジアの一部に限られていた。しかし、2007年に最初の土着のCHIKV伝播が欧州で報告された。高いレベルのウイルス血症、欧州や米国の大都市地域にも存在する媒介蚊、及び生理化学的な不活化工程に対するこのアルファウイルスの抵抗性についての不確実性は、血漿分画製剤の安全性についての関心を惹起した。
CHIKV に関する血漿製品の安全性の余裕を確認するために、普遍的に用いられるウイルス不活化工程（ヒト血清アルブミン（HSA）のための低温滅菌、活性を無視している第Ⅷ因子抑制剤のための蒸気加熱、静脈免疫グロブリン（IVIG）のための溶媒/界面活性剤（S/D）処理、IVIG のための低 pH インキュベーション）のこの新興ウイルス（CHIKV 及び密接に関連したシンドビスウイルス（SINV））に対する不活化効果を調べた。得られた結果は、西ナイルウイルスとよく使うモデルウイルス：ウシ下痢症ウイルスについて以前の研究と比較された。
生成されたデータは、評価した不活化工程が SINV と同様に CHIKV にも効果的な不活化効果があることを示し、それによってモデルウイルスが使われた初期のバリデーション研究からの結果を裏づけた。このことは血漿製品の安全に揺るがぬ安心を提供し、そしてこの結果は、新興ウイルスの物理化学的特性が良く特徴付けられている場合には、そのウイルスの不活化特性を予測するのにモデルウイルスの使用が適切であることを実証している。

使用上の注意記載状況・その他参考事項等

代表としてヘブスブリン IH 静注 1000 単位の記載を示す。
2. 重要な基本的注意
(1)本剤の原材料となる血液については、HBs抗原、抗HCV抗体、抗HIV-1抗体、抗HIV-2抗体陰性であることを確認している。更に、プールした試験血漿については、HIV-1、HBV及びHCVについて核酸増幅検査（NAT）を実施し、適合した血漿を本剤の製造に使用しているが、当該NATの検出限界以下のウイルスが混入している可能性が常に存在する。本剤は、以上の検査に適合した高力価の抗HBs抗体を含有する血漿を原料として、Cohnの低温エタノール分画で得た画分からポリエチレングリコール4000処理、DEAEセファデックス処理等により抗HBs人免疫グロブリンを濃縮・精製した製剤であり、ウイルス不活化・除去を目的として、製造工程において60℃、10時間の液状加熱処理及びウイルス除去膜によるろ過処理を施しているが、投与に際しては、次の点に十分注意すること。

報告企業の意見	今後の対応
チクングニアウイルス（chikungunya virus：CHIKV）は、トガウイルス科アルファウイルス属に分類される直径70nmのエンベロープを有する球状のRNAウイルスで、蚊（ヤブカ属のネッタイシマカやヒトスジシマカ）によって媒介される。万一、原料血漿にチクングニアウイルスが混入したとしても、BVDをモデルウイルスとしたウイルスクリアランス試験成績から、本剤の製造工程において不活化・除去されると考えている。	本報告は本剤の安全性に影響を与えないと考えるので、特段の措置はとらない。

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ヘブスブリン

Chikungunya virus and the safety of plasma products

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BACKGROUND: Chikungunya virus (CHIKV) outbreaks were previously restricted to parts of Africa, Indian Ocean Islands, South Asia, and Southeast Asia. In 2007, however, the first autochthonous CHIKV transmission was reported in Europe. High-level viremia, a mosquito vector that is also present in large urban areas of Europe and America, and uncertainty around the resistance of this Alphavirus toward physiochemical inactivation processes raised concerns about the safety of plasma derivatives. To verify the safety margins of plasma products with respect to CHIKV, commonly used virus inactivation steps were investigated for their effectiveness to inactivate this newly emerging virus.
STUDY DESIGN AND METHODS: Pasteurization for human serum albumin (HSA), vapor heating for Factor VIII inhibitor bypassing activity, solvent/detergent (S/D) treatment for intravenous immunoglobulin (IVIG), and incubation at low pH for IVIG were investigated for their capacity to inactivate CHIKV and the closely related Sindbis virus (SINV). The obtained results were compared to previous studies with West Nile virus and the commonly used model virus bovine viral diarrhea virus.
RESULTS: The data generated demonstrate the effective inactivation of CHIKV as well as SINV by the inactivation steps investigated and thereby support results from earlier validation studies in which model viruses were used.
CONCLUSION: High inactivation capacities with respect to CHIKV were demonstrated. This provides solid reassurance for the safety of plasma products and the results verify that the use of model viruses is appropriate to predict the inactivation characteristics of newly emerging viruses when their physicochemical properties are well characterized.

Chikungunya virus (CHIKV) was first isolated during an outbreak in Tanzania in the 1950s and has subsequently caused frequent outbreaks in Africa and in Asia.[1] In 2004, CHIKV spread from Kenya to the Western Indian Ocean Islands including the Comoros Islands, La Réunion, Mayotte, Mauritius, the Seychelles, and Madagascar and from there CHIKV emerged further in India and the Eastern Indian Ocean Islands, with millions of people infected.[2] Whereas the previous vector of CHIKV was *Aedes aegypti*, the primary vector in La Réunion and Mauritius was *Aedes albopictus*, the Asian tiger mosquito.[3] The adaptation of CHIKV to *A. albopictus* was due to a single amino acid substitution, which significantly increased CHIKV infectivity for this species,[4] that is widely endemic in urban areas of Europe and America.[5,6] Since 2006, CHIKV infections have been identified in an unprecedented number of travelers,[7] some of them returning with high-grade viremia to countries where competent vectors are present, which raises serious concerns with respect to a potentially global spread of the disease.[7] The first outbreak of autochthonously transmitted CHIKV in a temperate climate zone occurred in 2007 in the province of Ravenna

ABBREVIATIONS: BHK = baby hamster kidney; BVDV = bovine viral diarrhea virus; CHIKV = chikungunya virus; FEIBA = Factor VIII inhibitor bypassing activity; LOD = limit of detection; S/D = solvent/detergent; SINV = Sindbis virus; SSM = spiked starting material; $TCID_{50}$ = tissue culture infectious dose 50%; WNV = West Nile virus.

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TRANSFUSION **;**:**–**.

in northeastern Italy,[8] with four smaller clusters of local transmission detected up to 49 km away from the initial introduction by a presumably single individual.[9] Of 337 suspected CHIKV cases, 306 were examined and 217 were laboratory confirmed.[10] Later, autochthonous transmissions have also been reported from southeastern France.[11] As a consequence of these recent outbreaks, CHIKV fever is no longer considered a disease that is restricted to tropical countries, but has developed into a worldwide public health concern.[2]

While cases of CHIKV transmission via blood products or organ transplantation have not been reported so far, such transmission events are possible,[12] as during an epidemic the risk of viremic donations is substantial,[1] with approximately one in four CHIKV infections asymptomatic,[13] and the viral load during the acute phase of infection, which typically lasts for 2 to 4 days, as high as 10^9 copies/mL.[7] These facts have raised sufficient concerns about the safety of plasma products for the US Food and Drug Administration to include the subject in the agenda of a workshop.[14] Dedicated virus inactivation and removal steps are integrated into the manufacturing processes of all fractionated plasma products that are licensed by advanced regulatory authorities to ensure their safety.[15] These process steps have been validated for their effectiveness in inactivating a broad range of physicochemically diverse viruses, including relevant (e.g., human immunodeficiency virus, hepatitis A virus) and so-called model viruses.[16] One of these model viruses, bovine viral diarrhea virus (BVDV) of the *Pestivirus* genus (*Flaviviridae*) is like CHIKV, a lipid-enveloped RNA virus and similar in size. As BVDV as well as the closely related Flavivirus West Nile virus (WNV) have been demonstrated to be effectively inactivated by the virus reduction methods commonly used in the manufacture of plasma products,[17,18] high margins of safety are equally presumed for CHIKV. Experimental evidence for CHIKV itself was, however, not previously available. To provide adequate assurance of virus safety, particularly for the individuals who critically depend on plasma-derived therapies, as well as regulatory authorities who need to guarantee the safety of such products on the market, the present work investigated CHIKV inactivation experimentally. In addition, some of the inactivation methods were evaluated using Sindbis virus (SINV), a virus closely related to CHIKV, and also WNV, with the aim of generating further supportive evidence for the inactivation capacity of these dedicated virus inactivation steps with respect to CHIKV. The results confirmed the CHIKV inactivation behavior expected from earlier model virus data and thereby show that concerns for the safety of plasma products with respect to CHIKV are unwarranted.

MATERIALS AND METHODS

Viruses and cells

CHIKV strain "LR2006-OPY1" was originally isolated in 2006 from a 73-year-old patient returning from La Réunion, a French overseas department,[7] and was obtained from the Centre for Ecology and Hydrology (Oxford, UK). Virus identity was confirmed by partial sequencing and sequence alignment with the whole genome of strain "LR2006-OPY1" (GenBank Accession No. DQ443544), which showed greater than 99% sequence homology. CHIKV working stock was produced on Vero cells adapted to growth in serum-free medium and titrated on Vero cells (ECACC No. 84113001, European Collection of Cell Cultures, Salisbury, UK).

SINV, strain Ar-339 (ATCC VR-68), was propagated on Vero cells (ATCC CCL-81) or on baby hamster kidney (BHK-21 [C13]) cells (Lee Biomolecular, San Diego, CA). $TCID_{50}$ (tissue culture infectious dose 50%) titration of SINV was done on Vero cells, whereas BHK cells were used for plaque assay.

WNV was obtained from the liver of a snowy owl found dead in New York in 1999. The virus was isolated by filtration of the liver homogenate, passaged on Vero cells, and lyophilized ("Isolate 385-99"). The Isolate 385-99 was characterized by sequencing parts of the genome[19] and provided by Dr Robert E. Shope (University of Texas, Galveston, TX). WNV was propagated and titrated on Vero cells (ECACC No. 84113001).

Infectivity assays

Virus-containing samples were titrated on the respective indicator cells by $TCID_{50}$ assays (CHIKV, SINV, WNV) or by plaque assay (SINV) using serial 0.5 or 1 log dilutions of samples. The cytopathic effects induced by the viruses were evaluated after incubation of the cells with the samples for 3 days (WNV, SINV plaque assay), 5 to 6 days (SINV $TCID_{50}$ assay), or 7 days (CHIKV) at 36 ± 2°C. Virus concentrations were calculated according to the Poisson distribution (CHIKV, WNV) or according to Reed and Muench (SINV) and expressed as $\log(TCID_{50})$/mL. Virus concentrations determined with the plaque assay were expressed as plaque-forming units/mL. To lower the limit of detection (LOD) for the samples taken during CHIKV and WNV runs after 600 minutes of pasteurization, after 30 and 60 minutes of solvent/detergent (S/D) treatment as well as on Days 14 and 20 of low-pH treatment, large-volume titration (bulk) was performed.

Downscaled manufacturing processes for plasma derivatives

All virus inactivation steps investigated apply to manufacturing processes for Baxter Healthcare (Westlake Village, CA) products. Downscaled models were established to mimic the manufacturing process steps as closely as possible. Except for SINV-spiked runs, process variables were adjusted to worst case conditions to investigate robustness of virus inactivation. The equivalence of the laboratory models to the respective large-scale processes was demonstrated by comparing critical process and selected product variables. Temperature as a critical process variable for virus inactivation was monitored continuously in all processes investigated. Process intermediates obtained from manufacture were used as starting material and spiked at a ratio between 1:10 to 1:20 with virus stock suspensions. From the spiked starting material (SSM) samples were taken and titrated to verify the amount of virus added. Further samples were collected during and at the end of the virus inactivation processes and titrated immediately. Corresponding unspiked or mock-spiked samples were taken from control runs without virus and tested for their potential cytotoxicity for indicator cell lines and for their potential interference with the detection of low virus titers. Virus reduction factors (log) were calculated in accordance with regulatory guidelines.[16]

Pasteurization of human serum albumin

During manufacture, human serum albumin (HSA) is heat treated as solution in the final container at 60°C for 600 to 660 minutes. The heat treatment is done for different concentrations of HSA, ranging from 5% to 25%. In the downscale, the virus inactivation capacity of the heat treatment step was investigated by incubation of the CHIKV- or WNV-spiked albumin solutions at 58 ± 1°C for 600 minutes. To show the robustness of the obtained virus inactivation, pasteurization of HSA was done at low and the highest protein concentration, that is, 5 and 25%. SINV-spiked HSA was heated at 60 ± 1°C for 600 minutes, at a protein concentration of 25% only. To show the equivalence of the downscale to the manufacturing process, selected biochemical variables such as aggregate concentration, purity, transferrin, and α-1 acidic glycoprotein levels were monitored and compared to sample results from manufacture.

Vapor heating of anti-inhibitor coagulant complex, that is, Factor VIII inhibitor bypassing activity

During the manufacture of Factor VIII inhibitor bypassing activity (FEIBA), a freeze-dried bulk intermediate with an adjusted residual moisture of 7% to 8% (wt/wt) is heat treated in a first phase at 60°C for a minimum of 510 minutes, followed by a second phase at 80°C for a minimum of 60 minutes. For the downscaled runs, process intermediate was spiked with virus and freeze-dried in 3-mL aliquots (2-mL aliquots for SINV-spiked intermediate). The residual moisture was adjusted before heat treatment by the addition of water for injection, using the method of Karl-Fischer. Heat treatment of CHIKV and WNV was done at 59.5 ± 0.5°C for 505 minutes followed by 79.5 ± 0.5°C for 55 minutes and a residual moisture content of 7% (wt/wt) and 8% (wt/wt), respectively. SINV-spiked intermediate was adjusted to a residual moisture of 7% to 8% (wt/wt) after freeze-drying and was heat treated at 60 ± 0.5°C for 600 minutes followed by 80 ± 0.5°C for 60 minutes. FEIBA activity (clotting assay), Factor (F)II (clotting assay), and FX activity (chromogenic assay) were determined for the downscale intermediate before and/or after the vapor-heating process, and the results were compared to the respective values for intermediates from manufacture, to confirm equivalence of the different scale processes.

S/D treatment of intravenous immunoglobulin

For S/D treatment of intravenous immunoglobulin (IVIG) products Gammagard S/D and Gammagard Liquid/KIOVIG (Baxter), a mixture of tri-*n*-butyl phosphate, Octoxynol-9 and Polysorbate 80 (Merck, Darmstadt, Germany) was added to target concentrations of 0.3% (vol/vol), 1% (vol/vol), and 0.3% (vol/vol), respectively, for at least 60 minutes at 18°C to 25°C and a pH value of 5.2. Downscale runs for CHIKV were done at 18 ± 1°C for 57 to 60 minutes. Concentrations of S/D components for CHIKV (Gammagard Liquid/KIOVIG) and SINV (Gammagard S/D) spiked runs were adjusted to 50% compared to the standard manufacturing conditions and the kinetics of virus inactivation further investigated using only 10% of the standard S/D concentrations. To prevent further inactivation of virus by the S/D reagents after sample drawing, S/D-containing samples were diluted immediately 1:100 or 1:20 (CHIKV, 50 or 10% of nominal S/D concentration) or 1:10 (SINV) in cold cell culture medium. The amount of S/D reagents added was measured by weighing and the concentration of each S/D reagent in solution was measured by specific assays in unspiked control runs. Protein concentration, conductivity, and immunoglobulin (Ig)G concentration of the respective intermediates of the two process scales were measured and compared to support the equivalence of the downscale to manufacture.

Low-pH treatment of IVIG (Gammagard Liquid/KIOVIG)

During the Gammagard Liquid/KIOVIG manufacturing process, the final product is incubated at a low pH of 4.4 to 4.9 and at a temperature of 30°C to 32°C for 21 to 24 days. To investigate virus inactivation of the low-pH incubation step at laboratory scale, CHIKV-spiked process intermediate was incubated at 29 ± 1°C for up to 21 days. The pH, adjusted to 4.4 and 4.9, was monitored and incubation temperature continuously recorded. To prevent further

inactivation of virus after sample drawing, samples were diluted immediately 1:3.16 with cold cell culture medium. Selected biochemical variables such as molecular size distribution, gamma-globulin purity, and functionally intact IgG were determined and compared to results of the large-scale process to verify the equivalence of the two process scales.

RESULTS

For each of the four dedicated virus inactivation steps as performed in downscaled versions in these verification studies for virus inactivation, the results for selected biochemical variables were always equivalent to those of the respective samples obtained from the manufacturing scale processes (data not shown), thereby confirming validity of the different scale processes. Generally, conditions least favorable for virus inactivation were chosen.

Pasteurization of HSA

As a dedicated virus inactivation step, the final HSA product is pasteurized. Investigation of the virus inactivation capacity in downscale runs was done below the lower limits of temperature and incubation time specified for manufacture, and robustness of the heat treatment step was further shown through the use of HSA with protein concentrations at the extremes of the specified manufacturing range. CHIKV was completely and rapidly inactivated to below the LOD within 30 minutes of incubation at 58 ± 1°C in both 5 and 25% HSA (Fig. 1, Table 1). SINV, which like CHIKV belongs to the Alphaviruses, showed very similar inactivation kinetics and was also completely inactivated to below the LOD within 30 minutes of pasteurization at 60 ± 1°C using 25% HSA (Fig. 1B, Table 1). Significant reduction factors (Table 1) and similar inactivation kinetics were seen for WNV, with complete inactivation achieved after 30 minutes in 25% HSA and after 60 or 180 minutes in 5% HSA (Fig. 1, Table 1). Comparing the results of the current study to earlier published BVDV pasteurization data,[18] this model virus for small enveloped RNA viruses was one of the most resistant against inactivation through pasteurization. Whereas CHIKV, SINV, and WNV infectivity was already significantly reduced during the heating phase to target temperature, BVDV was more resistant to heating (Fig. 1) and a longer pasteurization period was necessary to inactivate the virus to below the LOD (120 and 60 minutes in 5 and 25% HSA, respectively; Fig. 1, Table 1).

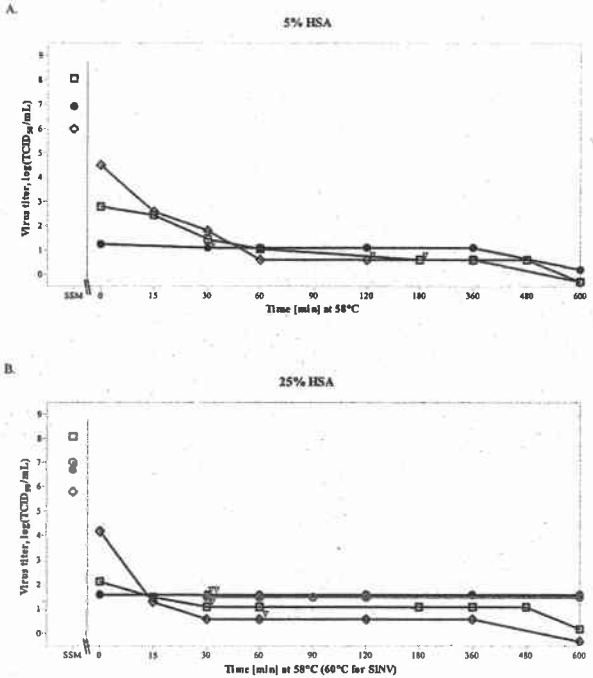

Fig. 1. Pasteurization of HSA. Virus inactivation kinetics for pasteurization at 58 ± 1°C (60 ± 1°C for SINV) of 5% HSA solution (A) and 25% HSA solution (B). Mean results for duplicate runs with CHIKV and WNV and single runs for BVDV and SINV are shown. SSM is virus-spiked HSA. For kinetic samples with no detected infectivity, the LOD is shown; (▽) first time point at which no infectivity was detected in all runs. Lower detection limits for virus titer toward the end of treatment is due to large-volume assays (bulk). (●) CHIKV; (◇) BVDV; (○) SINV; (□) WNV.

Vapor heating of FEIBA

The critical variables for the vapor heating process with respect to virus inactivation are temperature, time of heating, and residual moisture. FEIBA intermediate spiked with CHIKV or WNV was vapor heated at laboratory scale to the lower limit of temperature for just below the lower limit of the incubation time specified for the manufacturing process. To evaluate process robustness, the

TABLE 1. Reduction factors (log) and time points at which no remaining viral infectivity was detected (values in parentheses) in the current and previous inactivation studies

Process	CHIKV	SINV	WNV	BVDV*
Pasteurization of HSA,† 5%	>7.0/>6.9 (0-1/30 min)	ND	>8.3/>8.3 (60/180 min)	>6.4 (120 min)
25%	>5.9/>5.8 (0-1/30 min)	>5.5 (30 min)	>8.0/>7.7 (30/30 min)	>6.2 (60 min)
Freeze-drying/vapor heating of FEIBA,‡ 7%	>6.9 (360 min)	>5.3/>5.2/>4.7	>8.2 (360 min)	>5.6 (360 min)
8%	>7.3 (360 min)	(120/120/120 min)	>8.0 (360 min)	>5.6 (360 min)
S/D treatment of IVIG,§ 50%	>7.2/>6.7 (1-2/1-2 min)	>5.3 (1-2 min)	ND	>6.1/>6.2 (1-2/1-2 min)
10%	>7.2 (1-2 min)	>5.3 (1-2 min)	ND	ND
5%	ND	ND	>6.0‖/>5.9‖ (30/30 min)	>5.8 (1-2 min)
Low-pH incubation of IVIG,¶ pH 4.4	>7.9 (6 days)	ND	>5.5‖ (4 days)	>5.4 (14 days)
pH 4.9	>7.4 (14 days)	ND	>6.0/>6.1‖ (2/4 days)	>5.9/>5.6 (14/14 days)

* Data from Kreil et al.[18]
† Done using a solution with a concentration of 5 or 25% HSA.
‡ Done using product with residual moisture (wt/wt) of 7 or 8% except for SINV, where residual moisture was adjusted to 7% to 8%.
§ Done using 50, 10, or 5% of specified S/D chemicals.
‖ Data from Kreil et al.[17]
¶ Done using IVIG adjusted to a pH of 4.4 or 4.9, with the exception of WNV, for which solution adjusted to a pH of 4.5 was used.
ND = not done.

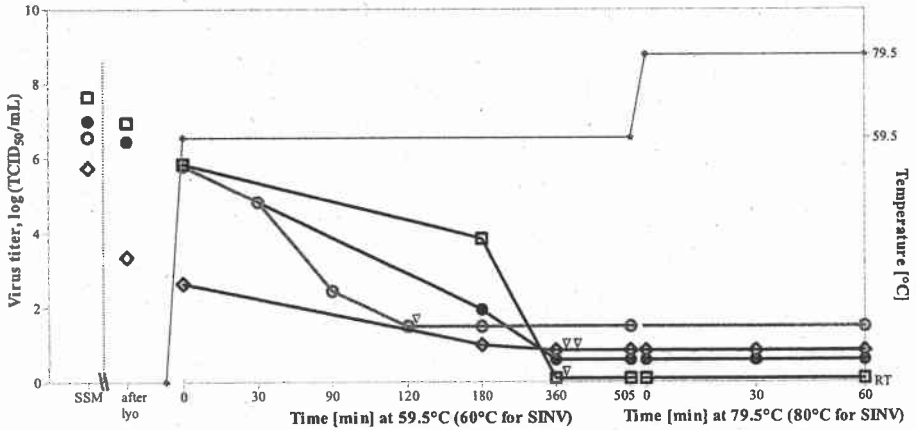

Fig. 2. Vapor heating of FEIBA. Virus inactivation kinetics by heating of virus-spiked, lyophilized, and moisture-adjusted (7%-8% [wt/wt]) FEIBA at 59.5 ± 0.5°C (60 ± 0.5°C for SINV), followed by 79.5 ± 0.5°C (80 ± 0.5°C for SINV) heating (temperature profile represented in graph by the gray line). Means of two samples per kinetic time point for all viruses except SINV (triplicates). The last SINV sample at 60 ± 0.5°C was taken after 600 minutes but shown in graph at 505 minutes for simplicity. SSM = virus-spiked FEIBA before lyophilization; "after lyo" = sample after lyophilization but before initiation of the heating process. For kinetic samples with no detected infectivity, the LOD is shown; (▽) first time point at which no infectivity was detected in all runs. RT = room temperature. (●) CHIKV; (◇) BVDV; (○) SINV; (□) WNV.

residual moisture was adjusted to the upper and lower limits of the manufacturing range. Using conditions least favorable for virus inactivation (temperature, time), the vapor heat treatment step in combination with the preceding freeze-drying inactivated CHIKV and WNV to below the LOD within 6 hours of the first heat treatment phase at a temperature of 59.5 ± 0.5°C (Fig. 2, Table 1). Heat treatment of SINV-spiked FEIBA intermediate at the same limits of temperature and residual moisture as specified during manufacture showed inactivation to below the LOD already after 2 hours in the first heat treatment phase (Fig. 2, Table 1). Comparing the results of the current study to earlier published BVDV vapor heating data,[18] efficient inactivation of the model virus was seen by this procedure, with no residual infectivity detected after 6 hours in the first phase of heat treatment, similar to

CHIKV and WNV (Fig. 2, Table 1). Interestingly, whereas the preceding lyophilization step had only a marginal effect on the infectivity of CHIKV, SINV, and WNV (Fig. 2), the infectivity of BVDV was more affected by this procedure, an observation that has been reported before.[20]

S/D treatment of IVIG (Gammagard Liquid/KIOVIG or Gammagard S/D)

At a final S/D concentration corresponding to only 50% of the manufacturing process, CHIKV and SINV were already inactivated to below the LOD after 1 to 2 minutes of S/D treatment (Fig. 3, Table 1). At 10% of the standard S/D concentration, CHIKV and SINV were also completely inactivated after 1 to 2 minutes of S/D treatment (Table 1), underlining the efficacy and robustness of the S/D treatment step. Comparing the results of the current study to earlier published BVDV S/D treatment data,[18] rapid and efficient inactivation of this model virus was seen at both 50 and 5% of the standard S/D concentration (Fig. 3, Table 1). In comparison, already available data for conditions deliberately chosen to be by far less stringent than the actual manufacturing process indicated that inactivation by S/D treatment was somewhat slower for WNV than for BVDV, as no infectious virus could be detected after 1 to 2 minutes of treatment with 5% of the standard S/D concentration for BVDV, but some low level of infectivity was detectable for WNV until 30 minutes of treatment (Fig. 3, Table 1).[17]

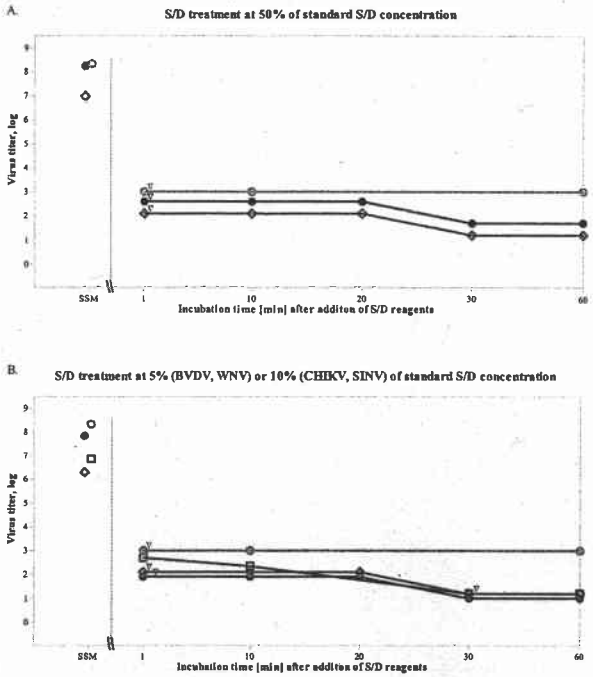

Fig. 3. S/D treatment of IVIG. Virus inactivation kinetics for 50% (A) and 5% or 10% (B) of standard S/D concentration for IVIG S/D treatment. Virus titers are log($TCID_{50}$/mL) for CHIKV, WNV, and BVDV and log(plaque-forming units/mL) for SINV. Mean results for duplicate runs with CHIKV and BVDV at 50% S/D concentration and WNV at 5% S/D concentration and single run results for all other experiments are shown. SSM = virus-spiked IVIG. For kinetic samples with no detected infectivity, the LOD is shown; (▽) first time point at which no infectivity was detected in all runs. Lower detection limits for virus titer toward the end of treatment is due to large-volume assays (bulk). (●) CHIKV; (◇) BVDV; (○) SINV; (□) WNV.

Low-pH treatment of IVIG (Gammagard Liquid/KIOVIG)

In the downscale investigation of the low-pH treatment step of the IVIG intermediate, CHIKV was inactivated to below the LOD on Day 6 for the run at pH 4.4 and on Day 14 for the run at pH 4.9 (Fig. 4, Table 1). Comparing the results of the current study to earlier published BVDV low-pH treatment data,[18] BVDV was similarly inactivated in the low-pH treatment, where by Day 14 complete inactivation was accomplished in all three experiments (Fig. 4, Table 1). In comparison, available data for WNV[17] showed that this Flavivirus was most rapidly inactivated by low-pH treatment. In one run at pH 4.9 WNV was already inactivated to below the LOD on Day 2 of incubation (Table 1). In the other two runs, one each at pH 4.5 and pH 4.9, no WNV could be detected in the first kinetic samples that were taken on Day 4 (Table 1). Complete and effective inactivation for all three viruses was therefore achieved at the two different extremes of the manufacturing specification for pH, demonstrating the robustness of the low-pH treatment step.

DISCUSSION

Over the past few years, the first autochthonously transmitted CHIKV infections have been reported in Europe,[8-11]

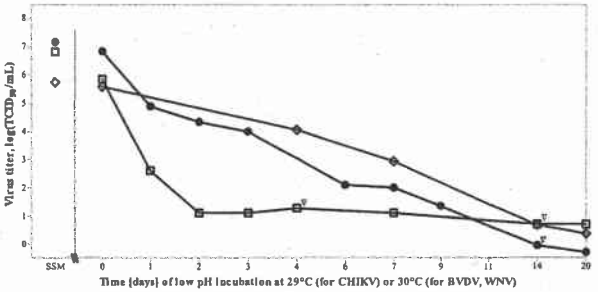

Fig. 4. Low-pH treatment of IVIG. Virus inactivation kinetics for incubation at pH 4.4 and pH 4.9 and 29 ± 1°C (CHIKV) or 30 ± 1°C (WNV, BVDV). Mean results of two runs (pH 4.4/pH 4.9) for CHIKV and three runs (pH 4.4/pH 4.9/pH 4.9) for BVDV and WNV (pH 4.5 instead of pH 4.4) are shown. SSM = virus-spiked IVIG. For kinetic samples with no detected infectivity, the LOD is shown; (▽) first time point at which no infectivity was detected in all runs. Lower detection limits for virus titer toward the end of treatment is due to large-volume assays (bulk). (●) CHIKV; (◇) BVDV; (□) WNV.

demonstrating that there is potential for the global spread of the virus[7] and that CHIKV has become a worldwide public health concern.[2] Similarly, spread into and subsequent autochthonous transmission of CHIKV would be entirely possible in the United States, where the mosquito vector *A. albopictus* is widely distributed in urban areas,[5,6] a scenario quite similar to the advent of WNV in the United States in 1999[21] and the epidemics since. Although reassuring data for the effective inactivation of small lipid-enveloped RNA viruses by the virus inactivation methods commonly used in the manufacture of plasma products exists,[17,18,22-25] this work sought to experimentally confirm the expected behavior for CHIKV, an Alphavirus and therefore a virus genus that is currently not typically used as a model virus.

CHIKV was subjected to virus inactivation methods commonly used in the manufacture of plasma products: pasteurization for HSA, vapor heating for FEIBA, S/D treatment for IVIG, and incubation at low pH for IVIG. In addition, some of the experiments were done with the closely related SINV and the relatively recent concern WNV, and the obtained results were compared to earlier published inactivation data for the model virus BVDV[18] and for WNV.[17]

Pasteurization of HSA resulted in effective inactivation of all the viruses tested, both at the low and at the upper protein concentration relevant in manufacture. It was shown that this method inactivated the Alphaviruses CHIKV and SINV more rapidly than BVDV and WNV.

During vapor heating of FEIBA, all the viruses investigated were already inactivated during the first heat treatment phase. Vapor heating was therefore confirmed as very effective, with the second heat treatment phase providing extra margins of safety. In addition, the results indicate that CHIKV, SINV, and WNV are more stable during lyophilization than BVDV (Fig. 2), confirming again the sensitivity of BVDV to this process step.[20] This is important to keep in mind should an investigation of virus inactivation for an isolated lyophilization step be desired, where BVDV as a model virus may not necessarily represent a worst-case choice of virus.

S/D treatment was again confirmed as being highly effective for the inactivation of all the viruses investigated.[25] Even only 5 or 10% of the S/D concentration specified for the manufacturing process was sufficient to rapidly and effectively inactivate all the viruses tested, and the results are thus in line with the original findings establishing this method as most effective in inactivating lipid-enveloped viruses.[26] Data from two different IVIG S/D treatment processes, Gammagard S/D and Gammagard Liquid/KIOVIG, are included as they use the same S/D reagents at identical concentrations. All available data show that there is no difference in efficacy of virus inactivation between these two IVIG S/D treatments when tested at process conditions as specified for manufacturing scale.

Complete and effective inactivation of all viruses investigated was obtained at the two different extremes of the manufacturing specifications for the low-pH treatment, where CHIKV and BVDV showed similar inactivation kinetics and WNV was most rapidly inactivated, an observation that is in line with the known acidic pH-dependent fusion machinery of Flaviviruses.[27] Comparing the resistance toward physicochemical inactivation across the different methods used in the manufacture of plasma products, CHIKV was very similar in susceptibility and virus inactivation kinetics to SINV, the other Alphavirus investigated, as well as to members of the Flaviviridae family, WNV and BVDV. Regarding the overall virus inactivation capacity, all the CHIKV spiked into experimental downscales was completely inactivated well before the end of these processes, indicating a great safety margin of these dedicated virus reduction techniques. Reassuringly, the inactivation methods tested were shown to completely inactivate all the virus that was spiked into the respective product intermediate and the data presented alleviate any CHIKV-associated concerns about the safety of plasma derivatives. Altogether, the CHIKV results obtained in this investigation provide solid reassurance of

the safety of plasma derivatives. In addition, our results verify that model viruses that are chosen according to suggestions of the relevant guidelines[16]—and if they are sufficiently well understood to be similar to transfusion-related viruses—are an adequate tool to predict the behavior of new viruses of interest.

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CONFLICT OF INTEREST

All authors are employees of Baxter BioScience: JM, GP, AB, MKH, PNB, and TRK have stock interests.

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販売名(企業名)	解凍赤血球濃厚液「日赤」(日本赤十字社) 照射解凍赤血球濃厚液「日赤」(日本赤十字社) 解凍赤血球-LR「日赤」(日本赤十字社) 照射解凍赤血球-LR「日赤」(日本赤十字社)					

研究報告の概要

○チクングニヤ熱アウトブレイク 中国広東省、2010年

2010年10月、中国広東省でチクングニヤ熱のアウトブレイクが報告された。

広東省東莞市の2つの隣接した村で173人の患者が確認された(2-93歳)。最初の患者は9月1日に発症し、9月19日以降、患者数が急増した。このアウトブレイクで死亡した患者はおらず、大部分の患者は発症してから1週間以内に回復した。患者やその家族の誰も、2010年7月以降に海外旅行をしていなかった。アウトブレイクの前月、広東省の天気は雨が多く、これにより媒介蚊の個体数が増加したと考えられる。

12人の急性患者及び3人の回復期患者から血清検体を採取し、このアウトブレイクを引き起こしているチクングニヤウイルスの系統発生解析を行ったところ、このチクングニヤウイルスは東/中央/南アフリカ(ECSA)サブグループのインド洋分岐群に属することが示された。

チクングニヤウイルスは2010年以前には中国の風土病ではなかったが、近年、東南アジアからのECSAジェノタイプに感染した患者から広東省に伝播したと考えられる。今回のアウトブレイクでは、症状がそれほど重篤でなかったこと及びデング熱との誤診により、ウイルス伝播が広範囲に及んだ。蚊の増加とチクングニヤウイルスの免疫保有者が少ないことが、このアウトブレイクに関連する要因となった。

使用上の注意記載状況・その他参考事項等

解凍赤血球濃厚液「日赤」
照射解凍赤血球濃厚液「日赤」
解凍赤血球-LR「日赤」
照射解凍赤血球-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
2010年10月、中国広東省でチクングニヤ熱のアウトブレイクが起こり、東莞市の2つの隣接した村で173人の患者が確認されたとのことである。	日本赤十字社では、輸血感染症対策として受付時に海外滞在歴の有無を確認し、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。

MedDRA/J Ver.15.0J

Chikungunya Outbreak in Guangdong Province, China, 2010

De Wu, Jie Wu, Qiaoli Zhang, Haojie Zhong, Changwen Ke, Xiaoling Deng, Dawei Guan, Hui Li, Yonghui Zhang, Huiqiong Zhou, Jianfeng He, Linghui Li, and Xingfen Yang

A disease outbreak with dengue-like symptoms was reported in Guangdong Province, China, in October 2010. Testing results confirmed that the pathogen causing the outbreak was chikungunya virus. Phylogenic analysis indicated that this virus was a member of the Indian Ocean clade of the East/Center/South African subgroup of chikungunya virus.

Chikungunya virus (CHIKV) is a mosquito-borne virus that causes fever, headache, rash, nausea, vomiting, myalgia, and arthralgia, and has had a major effect on human health (*1*,*2*). The first human infections caused by CHIKV were reported ≈60 years ago (1952–1953) in eastern Africa (3). CHIKV has now become a worldwide public health problem. Although this virus is indigenous to tropical Africa, outbreaks of CHIKV fever have been reported in countries in the Indian Ocean region and Southeast Asia (*4*–*6*). With an increase in global travel, the risk for spreading CHIKV to regions in which the virus is not endemic has increased (*7*).

Multiple sporadic cases of nonindigenous CHIKV infection have been reported in China. In 1987, CHIKV was isolated from the serum of a patient, and antibodies against CHIKV were detected in a second, convalescent-phase patient in Yunnan Province (*8*). Four imported cases of CHIKV infection confirmed by reverse transcription PCR (RT-PCR) were detected in Guangzhou and Morning, Guangdong Province, in travelers returning from Sri Lanka and Malaysia in 2008 (*9*,*10*). Another imported case from India was confirmed by using RT-PCR in our laboratory in 2009. We report an outbreak of CHIKV fever that occurred in Guangdong Province, China, in 2010.

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The Study

Guangdong Province is located in a subtropical zone. It has a high relative humidity, an average yearly temperature of 19°C–24°C, and an average yearly rainfall of 1,300–2,500 mm. *Aedes albopictus* mosquitoes are abundant and widespread. However, *Ae. aegypti* mosquitoes are found only in western Guangdong Province and not in the region around the city of Dongguan. In the months before the outbreak, the weather in Guangdong Province was particularly rainy.

During September 2010, patients reporting an illness with dengue-like symptoms were recorded by local community clinics in the suburbs of Dongguan, Guangdong Province. For epidemiologic investigation, the Guangdong Center for Disease Control and Prevention defined a clinical case of CHIK fever as a case characterized by sudden onset of fever with arthralgia, maculopapular rash, or myalgia. We identified 173 patients (74 male and 99 female patients) 2–93 years of age in 2 adjacent villages who had similar symptoms. More than 85% of the patients were found in these 2 villages in 97 families (≥2 cases per family in 50 families).

The first patient became ill on September 1, and the number of CHIKV fever cases rapidly increased after September 19 (Figure 1), indicating an outbreak of CHIKV infections in the region. The outbreak spanned 2 months, and the peak occurred at the end of September/early October. None of the patients or any family members reported travel abroad since July 2010. No deaths were reported as a result of the outbreak, and most patients recovered within 1 week after onset of symptoms. No patients were hospitalized; however, several elderly patients reported joint pain after 2 weeks.

Densities of *Ae. albopictus* mosquitoes were investigated during the outbreak, and an especially high Breteau index of 77–180 was observed. The abundant rainfall likely resulted in an extremely high mosquito density. To control the outbreak, mosquito control measures were implemented and quarantine of patients with acute disease was enforced.

To identify the pathogen causing the outbreak, we collected 15 serum samples from 12 patients with acute disease and 3 patients with convalescent-phase disease who had dengue-like symptoms. Patient serum was assayed for CHIKV nucleic acid, antibody, and virus. DNA sequence analysis of amplified CHIKV envelope 1 (E1) was performed to infer possible source of transmission. Specimens were tested by real-time RT-PCR for CHIKV (*11*) and dengue virus.

Ten serum samples were positive for CHIKV. Virus-specific IgM and IgG were detected by IgM and IgG capture ELISAs (IBL, Hamburg, Germany). Seven samples were positive for IgM and 1 sample was positive for IgG (Table).

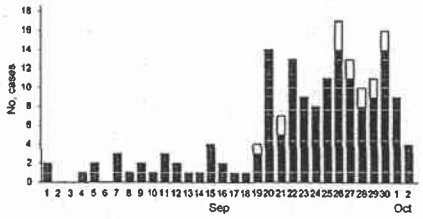

Figure 1. Cases of chikungunya infection in Guangdong, China, September 1–October 1, 2010. Black bar sections indicate clinical cases and white bar sections cases confirmed by molecular analysis.

There were 3 case-patients in whom CHIKV nucleic acid and antibody were found at the same time; 2 of these were in serum samples obtained 3–4 days after these samples were found to be positive for CHIKV IgM. We infer that high cross-reactivity in the ELISAs might contribute to these results.

For phylogenetic analysis, RT-PCR was performed as described (*12*), and 7 amplicons were sequenced. The 10 nucleic acid–positive specimens were placed on C6/36 and BHK-21 cell lines to isolate CHIKV. Serum samples were 2-fold serially diluted 6 times (1:50–1:1,600) in minimal essential medium, and 1 mL of diluted sample was added to each well of a 24-well culture plate. Specimens were incubated at 33°C in an atmosphere of 5% CO_2 and observed daily for ≤7 days for cytopathic effects (CPEs) (online Appendix Figure, wwwnc.cdc.gov/EID/article/18/3/11-0034-FA1.htm). After specimens were incubated for 4–7 days, 3 CPEs were observed on C6/36 and BHK-21 cells. Development of CPEs in C6/36 cells is unusual for CHIKV. However, we observed the effect of C6/36 cell fusion on 3 specimens. We speculate that a virus mutation causes an increase in virulence or changes effects on infected C6/36 cells.

Phylogenetic analysis was performed for partial E1 sequences (7 from this study and 24 from GenBank) by using MEGA5 (*13*). Nucleotide sequences were separated into 3 subgroups corresponding to the 3 globally circulating subgroups (Figure 2). Sequences of the 7 PCR products obtained in this study showed few differences from each other. Paired sequence identity ranged from 98% to 99% at the nucleotide level. Genetic analysis of the 325-nt fragment of E1 genes obtained in this study showed that all 7 sequences clustered in a unique branch within the Indian Ocean clade of the East/Central/South African (ECSA) genotype, and close to Thailand (GQ870312, FJ882911, GU301781), Malaysia (FJ998173), Taiwan (FJ807895), and China (GU199352, GU199353) isolates (98%–99%). The translated E1 gene fragment from 1 of the 7 isolates in this study (China/GD112/2010) had an expected 2-codon deletion. This deletion was also present in the ESCA clade but was not found in the other 6 isolates.

On the basis of sequence analysis, the highest degree of identity was observed with outbreak isolates and the E1 sequence from the Thailand strain (FJ882911) isolated in 2009. Paired identity values were 99% at the nucleotide level and 100% at the amino acid level. Nucleotide substitute analysis showed that a common nucleotide substitution was observed at partial E1 gene site 250 (T/C) in outbreak isolates and FJ882911. This substitution was not observed in other analyzed sequences from GenBank. These results suggested that the virus causing this outbreak was likely transmitted from a source in Southeast Asia and probably evolved from a strain that originated in Thailand.

Table. Characteristics of case-patients and serum sample detection for chikungunya virus, Guangdong, China, 2010*

Case-patient ID no.	Age, y/sex	Date of symptom onset, Sep 2010	Signs and symptoms						Test results		
			Fever	Red face	Headache	Arthralgia	Myalgia	MR	Virus isolation	Real-time RT-PCR/RT-PCR	IgM/IgG
D10112	33/F	27	+	–	–	+	+	+	–	+/+	–/–
D10113	7/M	29	+	+	–	+	+	+	+	+/+	–/–
D10114	62/M	30	+	+	+	+	–	–	+	+/+	–/–
D10115	48/F	30	+	–	–	+	–	+	+	+/+	–/–
D10116	60/M	28	+	–	–	+	+	–	–	+/–	–/–
D10117	39/M	27	+	+	–	+	+	+	–	+/–	+/–
D10118†	59/M	19	+	+	–	+	–	+	ND	–/ND	+/+
D10119	59/F	26	–	+	–	+	–	+	ND	–/ND	–/–
D10120	10/F	26	+	–	+	–	–	+	ND	–/ND	+/–
D10121†	56/F	21	+	+	–	+	–	+	ND	–/ND	+/–
D10122†	24/F	21	+	+	–	+	+	+	ND	–/ND	+/–
D10123	3/F	26	+	–	–	–	–	+	–	+/–	–/–
D10124	60/M	26	+	–	–	+	+	+	–	+/+	–/–
D10125	60/F	29	+	–	–	+	+	+	–	+/+	+/–
D10126	39/M	28	+	–	–	+	+	+	–	+/+	+/–

*All samples were obtained on October 1, 2010. ID, identification; MR, maculopapular rash; RT-PCR, reverse transcription PCR; +, positive; –, negative; ND, not done.
† Convalescent-phase case-patient.

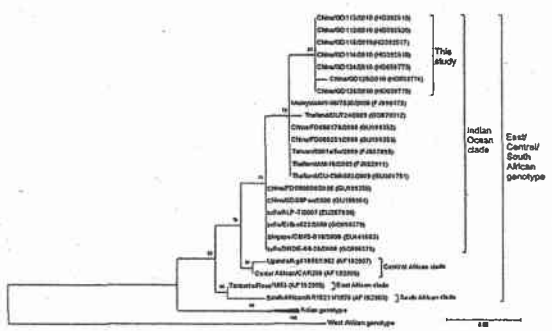

Figure 2. Phylogenetic analysis of partial envelope 1 gene sequences of chikungunya viruses, Guangdong, China, 2010. Numbers along branches indicate bootstrap values. GenBank accession numbers are indicated in parentheses. Scale bar indicates nucleotide substitutions per site.

Conclusions

CHIKV was not endemic to China before 2010. However, in recent years, CHIKV strains from Southeast Asia with the ECSA genotype have been transmitted by infected persons to Guangdong Province. We report an outbreak of CHIKV fever in China. The low severity of the disease and misdiagnosis of dengue fever has likely encouraged widespread transmission of the virus. High-density mosquito populations and an immunologically uninfected population were 2 contributing factors in this outbreak.

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医薬品
医薬部外品　　研究報告　調査報告書
化粧品

識別番号・報告回数			報告日	第一報入手日 2012 年 4 月 17 日	新医薬品等の区分 該当なし	厚生労働省処理欄
一般的名称	①②ポリエチレングリコール処理抗破傷風人免疫グロブリン ③乾燥抗破傷風人免疫グロブリン		研究報告の公表状況	CDC/Newsroom Home/Press Release/2012/03/14	公表国 アメリカ	
販売名 （企業名）	①テタノブリン IH 静注 250 単位　(ベネシス) ②テタノブリン IH 静注 1500 単位　(ベネシス) ③テタノブリン筋注用 250 単位　(ベネシス)					

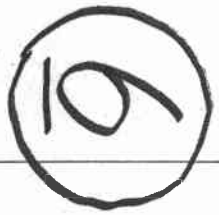
研究報告の概要

胃腸炎による死亡が 2 倍
C. difficile とノロウイルスが主要な原因である

疾病管理予防センター（CDC）による検査によれば、胃腸炎（嘔吐と下痢を引き起こす胃と腸の炎症）で死亡した人の数は、1999 年から 2007 年までに 2 倍以上になった。調査結果は、本日、アトランタでの新興感染症に関する国際会議で発表される。
CDC の科学者は、米国における全年齢層の間で 1999 年から 2007 年までの胃腸炎関連の死亡を特定するために、全国健康統計センターからのデータを使用した。
「胃腸炎は、世界的に死亡の主たる原因である」と CDC のウイルス疾病部門の執筆者アロン・ホール氏は言った。「胃腸炎関連の死亡の原因及び危険に曝されていることを知ることによって、我々はより良好な治療を開発することができ、医療従事者が人々が病気になるのを防ぐのを手助けすることができる。」
8 年間の調査期間で、全ての原因による胃腸炎関連の死亡は年間ほぼ 7,000 から 17,000 以上まで増加した。65 歳以上の高齢者が死亡の 83%を占めた。クロストリジウム・ディフィシール（C. difficile）とノロウイルスが、胃腸炎関連の死亡の最も多い感染原因であった。保健医療の現場にしばしば関連した細菌の一種、C. difficile のために、年間死亡数は約 2,700 から 14,500 まで、5 倍増加した。下痢を引き起こす C. difficile は、死亡の 2/3 を占めた。C. difficile の発生率と死亡率の最近の増加の多くは、過剰毒性、耐性株の C. difficile の出現と広がりに起因していた。
ノロウイルスは毎年約 800 の死亡と関連し、流行がウイルスの新しい株に起因した年には 50%以上多い死亡数となった。ノロウイルスは高い接触感染性がある。それはヒトからヒトへの接触と汚染された食品、水及び体表面を介して広がる。人々は年間を通してノロウイルスの病気に罹患するが、症例は 12 月から 2 月の間にピークに達する。ノロウイルスは毎年 2000 万以上の病気を引き起こす、そしてそれは米国での胃腸炎流行の主要な原因である。
「C. difficile が胃腸炎関連の死亡の主要な一因であり続けると同時に、本研究はノロウイルスがおそらく第二の主要な感染原因であることを初めて示す」とホール氏は言った。「我々の調査研究は、特に高齢者の間の C. difficile とノロウイルスによる胃腸炎を防止、診断し、管理するための有効な手段の必要性を強調する。」

使用上の注意記載状況・その他参考事項等

代表としてテタノブリン IH 静注 250 単位の記載を示す。
2. 重要な基本的注意
(1)本剤の原材料となる血液については、HBs 抗原、抗 HCV 抗体、抗 HIV - 1 抗体、抗 HIV - 2 抗体陰性であることを確認している。更に、プールした試験血漿については、HIV - 1、HBV 及び HCV について核酸増幅検査（NAT）を実施し、適合した血漿を本剤の製造に使用しているが、当該 NAT の検出限界以下のウイルスが混入している可能性が常に存在する。本剤は、以上の検査に適合した高力価の破傷風抗毒素を含有する血漿を原料として、Cohn の低温エタノール分画で得た画分からポリエチレングリコール 4000 処理、DEAE セファデックス処理等により抗破傷風人免疫グロブリンを濃縮・精製した製剤であり、ウイルス不活化・除去を目的として、製造工程において 60℃、10 時間の液状加熱処理及びウイルス除去膜によるろ過処理を施しているが、投与に際しては、次の点に十分注意すること。



別紙様式第 2-1
番号 9

医薬品
医薬部外品　　研究報告　調査報告書
化粧品

報告企業の意見	今後の対応	
クロストリジウム・ディフィシル（Clostridium difficile）は、大きさ0.5～1.9×3.0～16.9μmの偏性嫌気性のグラム陽性桿菌である。万一、原料血漿にC. difficileが混入したとしても、除菌ろ過等の製造工程において除去されると考えている。 また、ノロウイルス（Norovirus）は、カリシウイルス科（Caliciviridae）に属する直径：30～38nmの正二十面体（表面に32個のカップ状の窪みが見られる）のエンベロープを有しないプラス鎖の一本鎖RNAウイルスである。万一、原料血漿にノロウイルスが混入しても、EMC及びCPVをモデルウイルスとしたウイルスクリアランス試験成績から、本剤の製造工程において不活化・除去されると考えている。	本報告は本剤の安全性に影響を与えないと考えるので、特段の措置はとらない。	

Centers for Disease Control and Prevention
CDC 24/7: Saving Lives. Protecting People. Saving Money through Prevention.

Press Release

For Immediate Release: March 14, 2012
Contact :CDC Division of News and Electronic Media
(404) 639-3286

Deaths from gastroenteritis double

C. difficile and norovirus are the leading causes

The number of people who died from gastroenteritis (inflammation of the stomach and intestines that causes vomiting and diarrhea) more than doubled from 1999 to 2007, according to a study by the Centers for Disease Control and Prevention. The findings will be presented today at the International Conference on Emerging Infectious Diseases in Atlanta.

CDC scientists used data from the National Center for Health Statistics to identify gastroenteritis-associated deaths from 1999 to 2007 among all age groups in the United States.

"Gastroenteritis is a major cause of death worldwide," said lead author Aron Hall, D.V.M., M.S.P.H., of the CDC's Division of Viral Diseases. "By knowing the causes of gastroenteritis-associated deaths and who's at risk, we can develop better treatments and help health care providers prevent people from getting sick."

Over the eight-year study period, gastroenteritis-associated deaths from all causes increased from nearly 7,000 to more than 17,000 per year. Adults over 65 years old accounted for 83 percent of deaths. Clostridium difficile (C. difficile) and norovirus were the most common infectious causes of gastroenteritis-associated deaths.

There was a fivefold increase, from approximately 2,700 to 14,500 deaths per year, for C. difficile, a type of bacteria often associated with health care settings. C difficile, which causes diarrhea, accounted for two-thirds of the deaths. Much of the recent increase in the incidence and mortality of C. difficile is attributed to the emergence and spread of a hypervirulent, resistant strain of C. difficile.

Norovirus was associated with about 800 deaths annually, though there were 50 percent more deaths in years when epidemics were caused by new strains of the virus. Norovirus is highly contagious. It spreads through person-to-person contact and contaminated food, water, and surfaces. People can get norovirus illness throughout the year, but cases peaked between December-February. Norovirus causes more than 20 million illnesses annually, and it is the leading cause of gastroenteritis outbreaks in the United States.

"While C. difficile continues to be the leading contributor to gastroenteritis-associated deaths, this study shows for the first time that norovirus is likely the second leading infectious cause," said Hall. "Our findings highlight the need for effective measures to prevent, diagnose, and manage gastroenteritis, especially for C. difficile and norovirus among the elderly."

###

U.S. Department of Health and Human Services

CDC works 24/7 saving lives, protecting people from health threats, and saving money through prevention. Whether these threats are global or domestic, chronic or acute, curable or preventable, natural disaster or deliberate attack, CDC is the nation's health protection agency.

Historical Document: March 12, 2012

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別紙様式第２

医薬品　研究報告　調査報告書

識別番号・報告回数		報告日	第一報入手日 2012年2月14日	新医薬品等の区分 該当なし	厚生労働省処理欄
一般的名称	①②フィブリノゲン加第XIII因子 ③④人血液凝固第XIII因子	研究報告の公表状況	Transfusion-associated Babesiosis in the United States: a description of cases Annals of Internal Medicine 2011:155(8)509-519	公表国 米国	
販売名（企業名）	①ベリプラストP コンビセット ②ベリプラストPコンビセット組織接着用 ③フィブロガミンP ④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

【背景】バベシア症は赤血球内寄生虫による死に至る可能性がある疾病で、通常ダニ媒介により感染するが、輸血によっても感染する。米国では主に北東部および中西部の7州においてダニ媒介によるバベシア症が発生したが、血液ドナーに対するバベシア症検査は未認可である。【目的】本研究では、輸血によるバベシア症が初めて確認された1979年以降の米国における輸血関連バベシア症例データを検証した。【方法】1979年～2009年に輸血を受け、2010年までに輸血後感染バベシア症と診断され、輸血以外の感染経路のエビデンスが報告されておらず、ドナーの感染が検査で確認されている患者を対象とした。【結果】輸血関連バベシア症例162例（B. microti 159例、B. duncani 3例）が本研究の対象となった。B. microti 159例の年齢中央値は65歳であった。ほとんどの症例が赤血球関連であったが、4例は全血由来血小板関連であった。122例（77%）は2000年～2009年に発生し、138例（87%）は風土病とされる主な7州（マサチューセッツ、ニューヨーク、コネチカット、ミネソタ、ロードアイランド、ニュージャージー、ウィスコンシン）で発生した。B. duncani 3例は赤血球輸血関連で、ワシントン州で1例、カリフォルニア州で2例記録された。【結論】以上の結果から、輸血感染リスク軽減のためのドナー検査の対策を講じる必要性がある。バベシア症は、輸血後の原因不明の溶血性貧血や発熱を呈する疾患の鑑別診断に含まれるべきである。

使用上の注意記載状況・その他参考事項等

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報告企業の意見	今後の対応
バベシア症は赤血球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。

Annals of Internal Medicine | ORIGINAL RESEARCH

Transfusion-Associated Babesiosis in the United States: A Description of Cases

Barbara L. Herwaldt, MD, MPH; Jeanne V. Linden, MD, MPH; Elizabeth Bosserman, MPH; Carolyn Young, MD; Danuta Olkowska, MD; and Marianna Wilson, MS

Background: Babesiosis is a potentially life-threatening disease caused by intraerythrocytic parasites, which usually are tickborne but also are transmissible by transfusion. Tickborne transmission of *Babesia microti* mainly occurs in 7 states in the Northeast and the upper Midwest of the United States. No *Babesia* test for screening blood donors has been licensed.

Objective: To ascertain and summarize data on U.S. transfusion-associated *Babesia* cases identified since the first described case in 1979.

Design: Case series.

Setting: United States.

Patients: Case patients were transfused during 1979–2009 and had posttransfusion *Babesia* infection diagnosed by 2010, without reported evidence that another transmission route was more likely than transfusion. Implicated donors had laboratory evidence of infection. Potential cases were excluded if all pertinent donors tested negative.

Measurements: Distributions of ascertained cases according to *Babesia* species and period and state of transfusion.

Results: 159 transfusion-associated *B. microti* cases were included; donors were implicated for 136 (86%). The case patients' median age was 65 years (range, <1 to 94 years). Most cases were associated with red blood cell components; 4 were linked to whole blood–derived platelets. Cases occurred in all 4 seasons and in 22 (of 31) years, but 77% (122 cases) occurred during 2000–2009. Cases occurred in 19 states, but 87% (138 cases) were in the 7 main *B. microti*–endemic states. In addition, 3 *B. duncani* cases were documented in western states.

Limitation: The extent to which cases were not diagnosed, investigated, reported, or ascertained is unknown.

Conclusion: Donor-screening strategies that mitigate the risk for transfusion transmission are needed. Babesiosis should be included in the differential diagnosis of unexplained posttransfusion hemolytic anemia or fever, regardless of the season or U.S. region.

Primary Funding Source: None.

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For author affiliations, see end of text.
This article was published at www.annals.org on 6 September 2011.

Babesiosis is caused by intraerythrocytic parasites, which usually are tickborne but also are transmissible by transfusion (1–9). In the United States, 2 species—*Babesia microti* and *B. duncani* (formerly, the WA1-type parasite [10, 11])—have been associated with both transmission routes. The predominant zoonotic agent is the rodent parasite *B. microti*, which is transmitted by *Ixodes scapularis* ticks in expanding foci in the Northeast and upper Midwest of the United States, particularly during spring and summer (1–3, 12). The first described tickborne and transfusion-associated *B. microti* cases occurred in Massachusetts in 1969 and 1979, respectively (13–15); the first such *B. duncani* cases were in Washington in 1991 and 1994 (10, 16).

Regardless of the transmission route, *Babesia* infection can range from asymptomatic to severe, in part depending on host factors (for example, asplenia and advanced age). Clinical infection is characterized by hemolytic anemia and nonspecific flu-like symptoms (such as fever, chills, and myalgia). Complications can include multiorgan dysfunction, disseminated intravascular coagulation, and death (1–3, 6, 7). Although a history of babesiosis is an exclusion criterion for blood donation (1), persons who meet all eligibility criteria (for example, they feel well, are afebrile, and are not anemic) can have low-level parasitemia and remain infective for months, even longer than a year (1–6, 16, 17). No *Babesia* assay for screening donors has been approved by the U.S. Food and Drug Administration (FDA) (1).

Posttransfusion babesiosis has been increasingly recognized (5–9, 18–29). However, national data and perspective about the U.S. burden of cases have been lacking. The Centers for Disease Control and Prevention (CDC) led a collaborative endeavor to ascertain and compile data on U.S. posttransfusion cases identified during the 3 decades since the first described case in 1979 (14). Here we summarize the transfusion-associated *Babesia* cases that we ascertained, including their distributions by species, time, and place.

Methods

Data Sources

Since the 1960s, the CDC's Parasitic Diseases Laboratory has been a national reference laboratory for *Babesia* testing. The CDC is often contacted regarding diagnos-

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Appendix Figure
Conversion of graphics into slides

Context

Babesiosis, a parasitic infection transmitted through tick bites, can also be acquired via blood transfusion and may result in life-threatening disease. There is no U.S. Food and Drug Administration–licensed test to screen blood donors for *Babesia* infection.

Contribution

The risk for transfusion-associated *Babesia* infection may be increasing. Cases have occurred year-round and have been seen in states where *Babesia* species are not endemic.

Caution

Although the cases ascribed to transfusion undoubtedly represent a fraction of those that occurred, some tickborne cases inadvertently might have been included.

Implication

Improvements in the prevention and detection of transfusion-associated babesiosis are urgently needed.

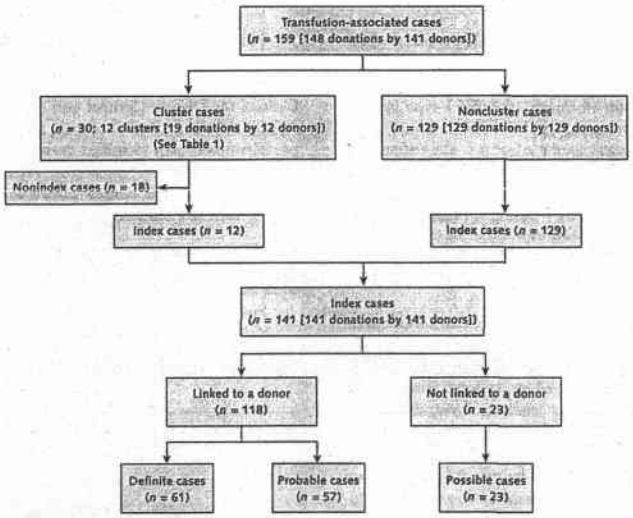
—*The Editors*

tic, clinical, and epidemiologic aspects of transfusion-associated and other *Babesia* cases. In addition to CDC records (such as records of test results, consultations, and case investigations), data sources for this endeavor included health departments, blood collection and transfusion services, other health professionals, and published materials and abstracts. The data available via health departments varied by jurisdiction and period; babesiosis was not a reportable disease in all states and was not nationally notifiable until January 2011. Although data were not systematically collected, some health departments, including those in babesiosis-endemic states, have routinely notified the CDC of potential transfusion cases and have submitted *Babesia* surveillance data to the CDC. Despite the inherent limitations of passive surveillance, collaborative relationships with public health and other pertinent agencies facilitated case ascertainment and data collection. We compiled and compared information obtained from multiple sources to maximize the quantity and quality of data and to minimize double counting.

Case Criteria and Classification

For these analyses, we established selection and classification criteria for transfusion-associated *Babesia* cases.

Figure 1. Stratification of 159 U.S. transfusion-associated *Babesia microti* cases, 1979–2009.



By type of case (cluster vs. not; index vs. not) and by class of index case (definite, probable, or possible). This figure, in conjunction with Table 1, provides perspective about the criteria for and the tallies of cases, donors, and donations. The 159 *B. microti* cases include 141 index cases and 18 nonindex, cluster cases. Each index case was associated with a different donor, whether implicated (n = 118) or virtual (n = 23; see Methods section). The 61 index cases classified as definite include the index cases for the 12 multicase clusters (Table 1), which encompass 18 additional cases, for a total of 79 cases. The 3 *B. duncani* cases are not included in the figure.

Table 1. Twelve Clusters of U.S. Transfusion-Associated *Babesia microti* Cases, 1979–2009*

Cluster	State (Year) of Transfusion	Case Type	Case Characteristics	Data on *Babesia* Case†	Comments About Recipients Other Than Case Patients‡
7 single-donation clusters§					
A	RI (2004)	Index	Preterm infant	Smear/PCR-positive	Another preterm corecipient of RBCs was treated empirically
		Corecipient	Preterm infant	Smear/PCR-positive	–
		Corecipient	Preterm infant	Smear/PCR-positive	–
B	RI (2006)	Index	Preterm infant	Smear-positive	No additional information
		Corecipient	Preterm infant	PCR-positive	–
		Corecipient	Preterm infant	PCR-positive	–
C	VA (2009)	Index	Preterm infant	Smear/PCR-positive	No other corecipients
		Corecipient	Preterm infant	Smear/PCR-positive	(Lookback: recipient of RBCs donated 3 mo earlier tested negative)
		Corecipient	Preterm infant	Smear/PCR-positive	–
D	NY (1997)	Index	Full-term infant	Smear/PCR-positive	Platelet corecipient (age 11 y) and 2 preterm corecipients of RBCs tested negative
		Corecipient	Preterm infant	Smear/PCR-positive	(Lookback >1 y earlier: RBC recipient tested negative; platelet recipient died ≤3 wk after transfusion)
		Corecipient	Age 70 y; GI bleeding	Smear/PCR-positive	–
E	NY (1999)	Index	Preterm infant	Smear/PCR-positive	Platelet corecipient reportedly was asymptomatic and was not tested
		Corecipient	Age 28 y; SCD	PCR-positive	(Lookback: "no adverse outcomes" reported for recipients associated with 2 previous donations)
F	CT (2006)	Index	Neonate	"Proven infection"	No additional information
		Corecipient	Age 32 y; SCD	"Proven infection"	–
G	MN (2008)	Index	Age 92 y; asplenic	Smear/PCR-positive	Double RBC donation: both recipients became infected and are listed here
		Corecipient	Age 36 y; surgery	PCR-positive	–
5 multidonation clusters‖					
H	MN (1999)	Lookback (July donation)	Age 78 y; GI bleeding	PCR-positive	Platelet corecipient (age 70 y) tested negative about 8 mo after transfusion
	MN (1999)	Lookback (September donation)	Age 80 y	PCR-positive	No corecipients
	MN (1999)	Index (November donation)	Age 68 y; surgery	Smear/PCR-positive	Platelet corecipient (age 81 y) tested negative about 6 mo after transfusion
	MN (2000)	Lookforward (January donation)	Age 67 y; surgery	Seropositive	RBC corecipient (age 73 y) died 2 d after transfusion
I	NY (2002)	Lookback (March donation)	Age 78 y; surgery	PCR-positive	(Further lookback: recipient associated with December 2001 donation tested negative)
	NY (2002)	Index (May donation)	Age 80 y; cirrhosis	Smear-positive	No corecipients
J	NY (2003)	Lookback (October donation)	Age 52 y; surgery	Seropositive	(Further lookback: no information about recipient of RBCs donated in August)
	MA (2004)	Index (December 2003 donation)	Age 74 y; carcinoma	Smear-positive	No additional information
K	WI (2007)	Lookback (August donation)	Age 83 y; surgery	Seropositive	(Status of other recipients of RBCs donated in 2007: 2 died; 1 tested negative; 1 lost to follow-up)
	FL (2008)	Index (February 2008 donation)	Age 83 y; GI bleeding	Smear/PCR-positive	No corecipients
L	MN (2008)	Index (August donation)	Age 61 y; leukemia	Smear/PCR-positive	No corecipients (lookback: RBC recipient associated with May donation tested negative)
	MN (2008)	Lookforward (October donation)	Age 53 y; surgery	Seropositive	No corecipients

CT = Connecticut; FL = Florida; GI = gastrointestinal; MA = Massachusetts; MN = Minnesota; NY = New York; PCR = polymerase chain reaction; RBC = red blood cell; RI = Rhode Island; SCD = sickle cell disease; VA = Virginia; WI = Wisconsin.

* The 12 identified clusters encompass 30 cases (1 per row) linked to 19 donations by the 12 implicated donors; the 30 cases include 12 index and 18 nonindex cases (11 in corecipients, 5 detected in lookback investigations, and 2 from lookforward investigations). One case was linked to whole blood–derived platelets (cluster H; fourth donation) (5); the other 29 were linked to RBC components. Among infants with available data, the smallest transfused volume was approximately 8 mL. In 2 multidonation clusters (J and K), case patients were identified in 2 states. In cluster J, both donations were in Maine, by a donor probably exposed in Massachusetts; in cluster K, a Wisconsin resident also donated in Florida. Five of 12 implicated donors had parasitologically confirmed infection, on the basis of testing an original unit segment (B, C, D, and G) or subsequent specimens (H); the donor linked to cluster H still had demonstrable parasitemia, by PCR analyses, 4 mo after the fourth donation, 10 mo after exposure (5). For cluster A's donor, a segment was available but results of PCR analyses were negative.

† "Seropositive" is noted only for the 4 nonindex cases that were not parasitologically confirmed: The reciprocal antibody titers ranged from 256 to 1024 in *B. microti* indirect fluorescent antibody testing.

‡ For recipients other than case patients, "tested negative" denotes seronegativity, at a minimum.

§ 18 cases (13 in infants and 5 in adults); 2–3 cases per cluster.

‖ 12 cases (all in adults); 1 case per donation; 2–4 donations per cluster.

Our minimal case criteria included receipt of 1 or more cellular blood components during 1979–2009, posttransfusion laboratory evidence of *Babesia* infection detected by 2010, and no reported evidence that another route of transmission (for example, tickborne or perinatal) was more likely than transfusion. We also required that linked (implicated) donors have laboratory evidence of infection. We excluded potential transfusion cases if all pertinent do-

Table 2. Characteristics of U.S. Transfusion-Associated *Babesia microti* Cases, Stratified by Type and Class (159 Total Cases, Including 141 Index Cases), 1979–2009*

Variable	All Cases (*n* = 159)	Stratification of All Cases, by Type (*n* = 159)	
		Index Cases (*n* = 141 [89%])	Nonindex Cases (*n* = 18 [11%])
Age at diagnosis, *n*	157	139	18
Median age (range; IQR), *y*†	65 (<1–94; 39–78)	66 (<1–94; 44–79)	34 (<1–83; <1–70)
Patients aged <1 y, *n (%)*†	18 (11)	11 (8)	7 (39)
Patients aged ≥1 y to <50 y, *n (%)*	33 (21)	30 (22)	3 (17)
Patients aged ≥50 y, *n (%)*	106 (68)	98 (71)	8 (44)
Male sex, *n/n (%)*	78/156 (50)	73/138 (53)	5/18 (28)
State of transfusion‡			
B. microti–endemic state, *subtotal n (%)*	138 (87)	122 (87)	16 (89)
Northeast (CT, MA, NJ, NY, or RI), *n*	118	108	10
Upper Midwest (MN or WI), *n*	20	14	6
Other state, *subtotal n (%)*	21 (13)	19 (13)	2 (11)
Eastern state, *n*	17	15	2
Not an eastern state, *n*	4	4	0
Year of transfusion			
Median (range)	2005 (1979–2009)	2005 (1979–2009)	2004 (1997–2009)
By period, *n (%)*			
1979–1984	4 (3)	4 (3)	0
1985–1989	3 (2)	3 (2)	0
1990–1994	6 (4)	6 (4)	0
1995–1999	24 (15)	19 (14)	5 (28)
2000–2004	31 (20)	26 (18)	5 (28)
2005–2009	91 (57)	83 (59)	8 (44)
Month of symptom onset or diagnosis, *n*§	–	128	–
Median (range)	–	Sep (Jan–Dec)	–
Interval from transfusion to diagnosis, *n*‖	–	114	–
Median (range; IQR), *d*	–	42 (14–230; 34–53)	–
Parasitologically confirmed infection, *n (%)*¶	153 (96)	139 (99)	14 (78)
Surgical splenectomy, *subtotal n***	32	32	0
History, *n*	17	17	0
Peritransfusion, *n*	12	12	0
≥1 mo after transfusion, *n*	3	3	0
Underlying condition or context for transfusion (1 per patient), *n*			
Hematologic disorder, *subtotal n*	39	37	2
Hematologic cancer	14	14	0
Sickle cell disease	11	9	2
Thalassemia major	7	7	0
Other hematologic disorder	7	7	0
Cardiovascular surgery or procedure	22	20	2
Gastrointestinal disease, bleeding, or surgery	19	17	2
Trauma with posttraumatic splenectomy**	8	8	0
Solid-organ transplantation††	5	5	0
Other surgery, procedure, or trauma	13	9	4
Newborn or complications of prematurity	16	9	7
Carcinoma	13	13	0
Other medical reason or diagnosis	14	14	0
Not specified	10	9	1
All-cause mortality, *n (%)*‡‡	28 (18)	27 (19)	1 (6)
Blood donor, *n (%)*§§	136 (86)	118 (84)	18 (100)
Parasitologically confirmed, *subtotal n*	24	24	–
PCR-positive unit segment, *n*	12	12	–

CT = Connecticut; IQR = interquartile range; MA = Massachusetts; MN = Minnesota; NJ = New Jersey; NY = New York; PCR = polymerase chain reaction; RI = Rhode Island; WI = Wisconsin.

* Data are number of cases/patients, unless otherwise noted. Diagnosis refers to babesiosis. Transfusion and blood donor refer to those associated with a case. Percentages might not total 100% because of rounding.

† Because a lower proportion of patients with index vs. nonindex cases were younger than 1 y ($P = 0.001$), the age distributions for index vs. nonindex patients were significantly different ($P = 0.009$), but not if the age comparison was limited to adults ($P = 0.3$).

‡ See Methods section and Figure 2. The "eastern state" category consists of Delaware, Florida, Indiana, Maryland, New Hampshire, North Carolina, Ohio, Pennsylvania, and Virginia. The "not an eastern state" category consists of California, Texas, and Washington.

§ If both were known and were different, the earlier month was specified. Data for the kidney donor (see text) were not included in analyses of month of diagnosis or interval to diagnosis.

‖ See Figure 3 regarding index patients. Among nonindex patients (Table 1), the interval to diagnosis depended on host factors, type of recipient (corecipient vs. other), and various aspects of the investigations. Although most of the ascertained nonindex patients who were adults reportedly were asymptomatic, clinical information in such regards typically was anecdotal or unspecified. In some investigations, other recipients could not be tested because they had already died.

Table 2—Continued

Stratification of Index Cases, by Class (*n* = 141)		
Definite Cases (*n* = 61 [43%])	Probable Cases (*n* = 57 [40%])	Possible Cases (*n* = 23 [16%])
60	56	23
69 (<1–94; 27–81)	65 (<1–92; 45–78)	67 (<1–87; 53–77)
9 (15)	1 (2)	1 (4)
11 (18)	16 (29)	3 (13)
40 (67)	39 (70)	19 (83)
25/60 (42)	33/55 (60)	15/23 (65)
44 (72)	57 (100)	21 (91)
–	–	–
–	–	–
17 (28)	0	2 (9)
–	–	–
–	–	–
2005 (1980–2009)	2006 (1979–2009)	2005 (1993–2009)
3 (5)	1 (2)	0
2 (3)	1 (2)	0
1 (2)	4 (7)	1 (4)
9 (15)	5 (9)	5 (22)
13 (21)	9 (16)	4 (17)
33 (54)	37 (65)	13 (57)
56	52	20
Aug (Jan–Dec)	Oct (Jan–Dec)	Sep (Jan–Dec)
53	50	11
43 (22–230; 35–52)	42 (14–225; 34–58)	42 (14–54; 21–52)
61 (100)	55 (96)	23 (100)
11	12	9
8	8	1
2	2	8
1	2	0
11	20	6
3	7	4
4	5	0
3	3	1
1	5	1
8	7	5
8	6	3
2	2	4
1	4	0
7	2	0
8	1	0
5	6	2
9	3	2
2	6	1
11 (18)	12 (21)	4 (17)
61 (100)	57 (100)	0
22	2	0
12	0	0

¶ Index cases were known or presumed to be parasitologically confirmed, with the exception of 2 cases classified as probable transfusion cases: the case in the kidney donor (see text) and a case diagnosed in retrospect, after recovery (30).

** The data constitute minimum numbers of case patients. Among the 12 known to have undergone splenectomy during the peritransfusion period, the contexts were trauma ($n = 8$) or abdominal surgery for other reasons ($n = 4$). The cases in the 3 patients known to have undergone posttransfusion splenectomy include 1 definite case (the index case of cluster L [25]; Table 1) and 2 probable cases, including the first described transfusion case (14).

†† Three received a kidney (living related [31], living unrelated, or cadaveric), 1 received a heart (29), and 1 underwent bilateral lung transplantation.

‡‡ Although outcome data were unavailable for some patients, we assumed that no other case patients died in the short term. The patients known to have died include 2 cluster-associated infants whose gestational ages were 23 and 24 wk, 2 (of 5) patients aged ≥90 y, and 6 (of 32) patients known to have undergone surgical splenectomy.

§§ In at least 4 case investigations, more than 1 donor had laboratory evidence of infection, typically 1 of whom was the most plausible on the basis of laboratory or epidemiologic data. However, the possibility of receipt of more than 1 contaminated unit could not be excluded.

nors tested negative. If multiple cases were linked to the same donor, we defined the interrelated cases as a cluster, the first identified case as the index case (1 per donor), and the other cases in the cluster as nonindex cases (Figure 1 and Table 1). To facilitate bookkeeping, we defined all cases that were not cluster-associated as index cases (1 per donor).

In general, index cases were parasitologically confirmed (Table 2) (30, 31); their detection prompted a transfusion investigation; and the linked donors and nonindex cases, if any, that were identified had parasitologic or serologic evidence of infection. We defined parasitologic evidence as detection of *Babesia* parasites (on blood smear or by animal inoculation) or *Babesia* DNA (by a molecular method). Serologic evidence of *B. microti* infection required positive results either by indirect fluorescent antibody (IFA) testing for total immunoglobulin or IgG or by immunoblot for IgG.

Index *Babesia* cases that fulfilled the selection criteria were classified as definite, probable, or possible transfusion-associated cases (Figure 1). If no donor was implicated among the subset of pertinent donors who could be tested, an index case was defined as a possible case, even if transfusion was the only known risk factor for infection. All index cases that were linked to a donor were classified as definite or probable cases. An index case was defined as a definite (vs. probable) transfusion case if at least 1 of the following additional criteria was fulfilled: 1) Transfusion was the only known or plausible risk factor for infection (for example, there was no history of residence or travel in babesiosis-endemic areas); 2) a multicase cluster was identified, with at least 1 nonindex case besides the index case (Table 1); 3) the linked donor's infection was parasitologically confirmed by testing an extant segment from the original blood unit; or 4) other donor evidence indicated active infection at the time of donation (for example, a polymerase chain reaction [PCR]–positive specimen that reflected the donor's status at donation).

Data Analysis

We conducted univariate analyses for descriptive purposes by using Epi Info, version 3.5.1 (CDC, Atlanta, Georgia), and SAS software, version 9.2 (SAS Institute, Cary, North Carolina). Proportions were compared by using the chi-square test, or if expected cell counts were less than 5, the Fisher exact test. The Wilcoxon 2-sample test was used to compare the ranked distributions of ordinal variables. Statistical significance was defined as a 2-sided P value less than 0.05.

Unless otherwise specified, we stratified cases by period and state of transfusion (Table 2 and Figure 2) (32). We refer to 7 states with well-established foci of zoonotic transmission as "*B. microti*–endemic states": 5 states in the Northeast (Connecticut, Massachusetts, New Jersey, New York, and Rhode Island) and 2 in the upper Midwest (Minnesota and Wisconsin) (1, 12). The distinction between these and other states (for example, in Figure 2) is not meant to imply that tickborne transmission occurs throughout these 7 states, that it occurred in all 7 states throughout 1979–2009, or that these are the only states in which it did or does occur. Of note, during case selection and classification, we considered the evolving focality of tickborne transmission within and among states.

Role of the Funding Source

The study received no external funding.

RESULTS

General Perspective and Summary

For the period of 1979–2009, we included 162 transfusion-associated cases: 159 *B. microti* cases and 3 *B. duncani* cases, which are described separately. The 159 *B. microti* cases include 12 multicase clusters encompassing 30 cases: 12 index cases (1 per cluster) and 18 nonindex cases (5, 8, 9, 20–25) (Figure 1 and Table 1). In total, 141 *B. microti* cases were defined as index cases: the 12 cluster-associated index cases and 129 additional cases (Table 2). Figure 2 shows their distribution by period and state of transfusion. During the initial 11 years (1979–1989), 7 index cases occurred in 5 states (14, 17, 32–36). In contrast, during the third decade (2000–2009), 109 index cases (77% of 141) and 122 total cases (77% of 159) occurred in 18 states (5–9, 18–21, 24–29, 37–42). The associated blood donations occurred in all 12 months (Appendix Figure, available at www.annals.org); 59% were during July–October.

Overall, 122 (87%) of the index cases (138 total cases [87%]) were associated with transfusions in the 7 main *B. microti*–endemic states (Figure 2 and Table 2), although not necessarily in areas of endemicity. The other 19 index cases (13%) generally were attributable to interstate movements of donors or blood components (Figure 2). Various scenarios are exemplified by the 4 cases not in eastern states (Table 2), 2 of which were attributable to donor travels: A Rhode Island resident donated while training in Washington (26), and a Texas resident donated in that state after spending the summer in Massachusetts (6). In contrast, the other case in Texas and the case in California were linked to donations in New Jersey and Maine (27), respectively. Local distributions of components collected in New Jersey also accounted for 2 cases in Pennsylvania (8, 37) and 1 in Delaware (18).

Case Characteristics

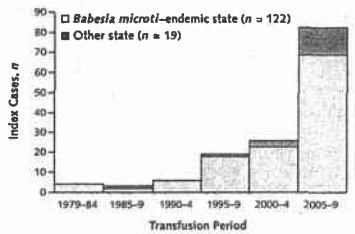
Table 2 summarizes selected characteristics of the cases, stratified by type of case (index vs. nonindex) and by class of index case (definite, probable, or possible). Table 1 provides additional perspective on the cluster-associated cases, which necessitated distinguishing between index and nonindex cases. Overall, the case patients had a median age of 65 years; 32% were either very old (33 were in the ninth or tenth decade of life) or very young (18 were infants, 13 of whom were cluster-associated). The 19 patients with hereditary blood disorders account for 73% of the 26 patients in the age range of 4 to 43 years. These 19 patients include 11 with sickle cell disease (8, 9, 28), 7 with thalassemia major (35, 43), and 1 with Diamond–Blackfan anemia (18); they account for at least 9 of the 32 patients known to have undergone surgical splenectomy. Three elderly patients with hematologic disorders underwent posttransfusion splenectomy (32, 134, or 215 days later), and their *Babesia* cases were diagnosed thereafter (Figure 3 and Table 2). For 2 of these patients, parasites were noted during retrospective review of presplenectomy blood smears, a finding that refocused the investigations on earlier transfusions and donors than on those initially targeted.

Five patients with transfusion cases had been transplanted with solid organs within the previous 3 months (Table 2). In addition, indirect evidence suggests that a kidney donor who received multiple transfusions the day he died served as a conduit of *Babesia* parasites from 1 of his blood donors to both of his kidney recipients, who developed parasitologically confirmed infection (40). No *B. microti* antibodies were detected by IFA testing of archived pretransplantation serum from the kidney recipients or of pretransfusion serum from the kidney donor (Table 2). However, postdonation specimens from 1 of his blood donors were seropositive (24).

The median interval from transfusion to onset of clinical manifestations was 37 days (range, 11 to 176 days) among 84 index patients with available data (Figure 3). Although babesiosis generally is considered a febrile illness, 13 (of 105) index patients were afebrile (9, 26, 32), including at least 4 adults who had cancer or were receiving immunosuppressive therapy. The median interval from symptom onset to diagnosis of index cases was 6 days (range, 0 to 54 days; $n = 84$). Babesiosis often was diagnosed incidentally, in some instances during routine outpatient evaluations (6), during hospitalizations for unrelated reasons, or after the patient had recovered (30) or died (data not shown). Typically, *Babesia* parasites were an unexpected finding when a blood smear was examined, usually in the context of a complete blood count with a manual differential (9). When intraerythrocytic ring forms were noted, malaria was the first diagnostic consideration for more than 20 index patients, at least 14 of whom were initially treated for malaria.

The minimum all-cause mortality rate among index patients was 19% (6–9, 18, 19, 32–34, 40, 44) (Table 2); Figure 3 provides various intervals to death. Some patients had a bleak prognosis even without the potential compounding effects of babesiosis. The 27 index patients known to have died include the kidney donor described earlier, whose posttrauma death on the day of transfusion clearly was unrelated to babesiosis. For other patients with available data, there was a spectrum of likelihood that

Figure 2. Distribution of U.S. transfusion-associated *Babesia microti* index cases, 1979–2009.



Cases, *n*	1979–84	1985–9	1990–4	1995–9	2000–4	2005–9
Endemic states*						
Massachusetts	2				2	12
New York	2			7	10	26
Connecticut		1	4	7	1	6
Minnesota		1	1	1		8
Rhode Island			1	1	8	11
New Jersey				2	1	4
Wisconsin					1	2
Other states†						
New Hampshire‡		1				
Maryland§				1		2
Pennsylvania					1	2
Texas					1	1
Washington					1	
Ohio‖						2
Indiana‖						1
Delaware						1
North Carolina						1
California						1
Florida						2
Virginia§						1
Total index cases per period	4	3	6	19	26	83

By period and state of transfusion. The data are limited to the 141 *B. microti* index cases, 12 of which were associated with multicase clusters (Table 1). Data for the 3 *B. duncani* cases, which occurred in Washington (in 1994) and California (in 2000 and 2008), are not included. The *x*-axis includes one 6-year period (1979–1984), followed by five 5-year periods. See the Methods section for the distinction between the 7 main *B. microti*–endemic states and "other states"; within each category, for the tallies by state (by period), the states generally are listed in the order of their first identified case.

* Local and intraregional movements of donors and blood components were common both in the Northeast and in the upper Midwest (data not shown).

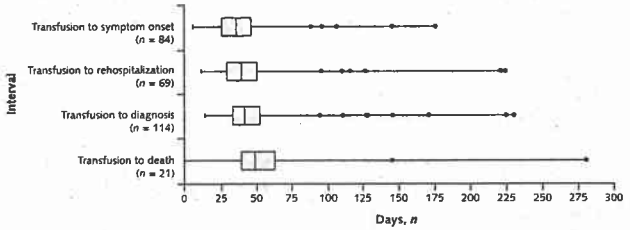
† Among the 19 index cases in 12 "other states," the North Carolina case and 1 Florida case were not linked to donors, the other Florida case was linked to a Wisconsin resident who donated blood while wintering in Florida (cluster K in Table 1), and 1 of 3 Pennsylvania cases was linked to a Pennsylvania donor who reportedly had not traveled to a known *B. microti*–endemic area in another state (8). Information on the donors linked to the other 15 index cases is provided in the text or the footnotes below for 7 and 8 cases, respectively.

‡ The donor was exposed in Massachusetts (32).

§ The 4 index cases in Maryland and Virginia were linked to donations in these states. The linked donors either were or could have been exposed in the Northeast.

‖ The cases in Ohio ($n = 2$) and Indiana ($n = 1$) were linked to donations in Indiana ($n = 2$) and Ohio ($n = 1$) by donors exposed in *B. microti*–endemic states.

Figure 3. Box-and-whisker plots of the distributions of time from transfusion to various events for U.S. transfusion-associated *Babesia microti* index cases, 1979–2009.



The data are limited to the subsets of the 141 index patients for whom particular intervals were relevant and were known or estimable (for example, the incubation period was unclear for some patients who had comorbid conditions or altered mental status). Each box represents the interquartile range (IQR), the internal vertical line indicates the median, the whiskers show the minimum and maximum, and the dots indicate the outliers with the longest intervals (≥75% quartile plus 1.5 × IQR). The 21 total dots—5 (6%), 6 (9%), 8 (7%), and 2 (10%) for the first, second, third, and fourth intervals from the top, respectively—are accounted for by 8 case patients, all of whom were linked to a donor. The farthest outliers include a patient with sickle cell disease who received hematopoietic progenitor cells from a sibling with sickle trait and became symptomatic approximately 6 months after the implicated peritransplantation transfusion (28) and 2 of 3 patients who underwent posttransfusion splenectomy (see text). The second interval from the top reflects the posttransfusion hospitalization during which babesiosis was explicitly diagnosed, for patients who had been discharged at least once in the interim or had been transfused as outpatients but were hospitalized thereafter. The fourth interval includes data for 21 of 27 index patients known to have died, including the kidney donor who died on the day he was transfused (see text). This interval was greater than 90 days for 2 immunocompromised patients whose intervals from diagnosis to death were less than 60 days. The patient who underwent splenectomy 215 days after transfusion died 280 days after transfusion; the patient's lymphoma also relapsed. For patients with available data, the median interval from symptom onset to death was 10 days (range, 2 to 51 days; $n = 18$) and the median interval from diagnosis to death was 7 days (range, 0 to 55 days; $n = 22$).

babesiosis had a causal or contributory role (6, 7); causes of death often were presumptive or unclear (data not shown).

Blood Donors and Components

A linked donor with laboratory evidence of *B. microti* infection was identified for 118 index cases (84%), which encompass 136 total cases (86%) (Figure 1). Among the 117 linked donors whose *B. microti* IFA test results were known, the median reciprocal antibody titer was 256 (range, 64 to 4096; interquartile range, 256 to 1024). Twenty-four donors (20%) had parasitologically confirmed infection (Table 2). The 20 donors with positive PCR results include 12 (71%) of 17 for whom blood retained from the original donation was tested compared with 8 (14%) of 56 for whom only postdonation specimens were available ($P < 0.001$). The median age of the 80 donors with available data was 49 years (range, 17 to 72 years); 18 donors (23%) were at least 60 years of age. Although clinical information typically was anecdotal or unspecified, some donors had pre- or postdonation symptoms or anemia of potential relevance (5, 24–27). For example, the donor who had 4 consecutive donations linked to transmission (cluster H in Table 1) had been temporarily deferred because he was anemic when he first attempted to donate after exposure (5).

Among the 151 cases for which the type of blood component was determined, 4 cases were linked to whole blood–derived platelets (4, 5, 14) and 147 were associated with red blood cells (RBCs). The median age of liquid-stored RBCs at the time of transfusion was 16 days (range, 4 to 40 days; $n = 106$); 4 case patients received RBCs that were 35 to 40 days old. At least 4 patients received frozen-deglycerolized (vs. liquid-stored) RBCs (18, 35, 43). Many patients received leukoreduced RBCs (data not shown); at least 10 received irradiated RBCs.

Babesia duncani Cases

The 3 documented *B. duncani* cases were linked to RBC transfusions in Washington (in 1994 [16]) and California (in 2000 [45] and 2008). In each instance, the case patient and implicated donor lived in the same state and had parasitologically confirmed infection. The case patients include a preterm infant (45), a 59-year-old man with a hemoglobinopathy (Bloch EM, Herwaldt BL, Leiby DA, et al. Unpublished data), and a 76-year-old man with a myelodysplastic syndrome who underwent cardiac surgery (16).

Discussion

Babesiosis is an uncommon but potentially life-threatening complication of transfusion that has been increasingly recognized since the first described U.S. case in 1979. Donor-screening practices do not yet include routine testing for evidence of *Babesia* infection. In this context, prompt detection, treatment, investigation, and reporting of *Babesia* cases are essential. Babesiosis should be included in the differential diagnosis of unexplained posttransfusion hemolytic anemia, with or without fever, regardless of the season or U.S. region. To enhance the ability of public health authorities to detect, monitor, and prevent transfusion and tickborne cases, babesiosis has been designated a nationally notifiable condition, effective January 2011; as such, cases reported to health departments are notifiable to the CDC.

For the 31-year period of 1979–2009, we included 159 *B. microti* transfusion-associated cases, which were dispersed in time (all 4 seasons and 22 years) and place (19 states). Protracted parasitemia in some infected donors (5, 6, 16, 17), donor travels to and from areas of endemicity (6, 26), and distributions or shipments of blood components account for the potential for year-round transmission anywhere in the country. Donor travel also accounted for the 1 reported transfusion-associated case of babesiosis in Canada, which was linked to a Canadian donor infected during a camping trip in Massachusetts (46). The majority (87%) of the 159 identified U.S. cases occurred in the 7 main *B. microti*–endemic states, which probably reflects higher risk and greater awareness. The annual case counts fluctuated, both overall and by locale (data not shown); the limited available risk estimates for transfusion transmission also have varied in time and place (2, 3, 8, 9, 30, 39). Even so, that the majority (77%) of these 159 cases occurred during 2000–2009 is noteworthy, regardless of whether some of the aggregate increase reflects improved recognition and reporting. In comparison, for the period of 1979–2009, the CDC's National Malaria Surveillance System tallied 49 cases of transfusion-associated malaria, only 5 of which occurred during 2000–2009 (Arguin P. Personal communication). *Babesia microti* has become the most frequently reported transfusion-transmitted parasite in the United States (2, 3). In general, public health reports of tickborne *Babesia* cases also have increased in aggregate, with temporal and spatial fluctuations (CDC. Unpublished data); a national surveillance definition was first implemented in January 2011.

In addition to the 159 *B. microti* cases, we included 3 *B. duncani* cases in western states (16, 45), for a total of 162 transfusion-associated cases. The *B. duncani* cases, like those caused by *B. microti*, were in patients who ranged from preterm to elderly and who had comorbid conditions. That infection with *B. duncani*—and with other U.S. zoonotic *Babesia* agents described since the 1990s (47, 48)—is not detected by serologic or molecular assays for *B. microti* has implications for diagnostic testing, transfusion investigations, and potential future donor screening.

As expected, almost all cases for which the type of component was determined were associated with RBC transfusions. Red blood cell components of all storage ages, including greater than 5 weeks, were associated with transmission, as were components that had been leukoreduced, irradiated, or frozen. Although we did not conduct risk analyses, our findings underscore that *Babesia* parasites can survive blood bank procedures and storage conditions for RBC components. The 4 identified cases linked to whole blood–derived platelets span from 1979 (the first described transfusion case) to 2000 and presumably were attributable to residual RBCs or to extracellular parasites in the platelet units (4, 5, 14, 49). These 4 cases—and the cases in infants transfused with small RBC aliquots—underscore that small inocula can suffice to cause infection. However, even a segment from an implicated unit may test negative by PCR: The small volumes tested do not approximate the volumes transfused (1, 2).

Some of the demographic and other characteristics of the case patients reflect those of transfused patients in general (2, 4) but may have particular importance in the context of babesiosis. For example, advanced age is a risk factor for severe babesiosis, even in otherwise healthy persons; transfusion recipients often have comorbid conditions that can increase their vulnerability to the compounding effects of babesiosis and interrelated complications (such as multiorgan dysfunction and death) (6, 7, 18, 19, 33, 34). On the other hand, even some of the adult index patients were afebrile, including several patients receiving immunosuppressive therapies that may affect the host response to infection. Although most index cases with available data were diagnosed within 2 months of transfusion, a noteworthy minority of cases were diagnosed months later, such as in the context of posttransfusion splenectomy (Figure 3). These points not only have clinical relevance but also may affect transfusion investigations and case counts: The likelihood that transfusion transmission is considered and is investigated successfully may be lower for cases with longer intervals from the pertinent transfusion to symptom onset or diagnosis.

The 162 transfusion-associated cases we enumerated undoubtedly represent a fraction of those that occurred. The extent to which cases were not detected, investigated, or reported (to the CDC, to other public health authorities, or in publications) is unknown, both in general and with respect to periods, regions, and various case characteristics and outcomes. As underscored by the incidental diagnosis of *Babesia* infection, even severe cases in babesiosis-endemic regions can be missed or misdiagnosed, not just cases that are asymptomatic or mild or that occur in other U.S. regions. Even if a case is diagnosed, a transfusion investigation might not be considered, conducted, completed, or conclusive. The cases we included that were not linked to a donor (Figure 1 and Table 2) highlight the challenges associated with contacting all pertinent donors and obtaining posttransfusion specimens for testing; segments from the original donations typically are not still available. Our tallies probably constitute undercounts even of documented transfusion cases (for example, those that did not come to our attention or did not meet our selection criteria) but inadvertently might include some tickborne cases. As with all surveillance, case ascertainment, selection, and classification depended on the completeness and accuracy of the available data.

Our findings underscore the year-round vulnerability of the U.S. blood supply—especially, but not only, in and near babesiosis-endemic areas. They also highlight the importance of multiagency collaborative efforts to detect, investigate, and document transfusion cases; to assess the risks for transfusion transmission; and, thereby, to inform the scope of prevention measures. In 2009, the Transfusion-Transmitted Diseases Committee of AABB (formerly, the American Association of Blood Banks) categorized babesiosis in the highest risk level for blood safety to be prioritized for intervention (50). Donors with subclinical infection are not identified by existing measures (such as temporary deferral of persons with systemic symptoms, fever, or anemia), no *Babesia* assay for screening donors has been approved by the FDA, and pathogen reduction techniques for RBCs or platelets are not available in the United States (1, 2, 50). The FDA's Blood Products Advisory Committee that was convened on 26 July 2010 supported the concept of regional donor testing for *Babesia* (51). The increasing recognition of transfusion cases strengthens the impetus for screening strategies that mitigate the transmission risk (1–3, 50, 51), including testing approaches implemented under FDA-approved protocols (1, 3, 51) and longer-term strategies with development of a high-throughput *Babesia* screening assay.

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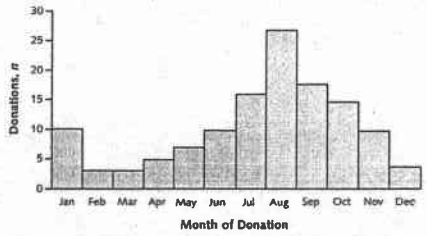
Collection and assembly of data: B.L. Herwaldt, J.V. Linden, C. Young, D. Olkowska, M. Wilson.

Appendix Figure. Distribution by month of the blood donations associated with U.S. *Babesia microti* transfusion cases (n = 128 of 148 total donations), 1979–2009.



The month of donation was known or estimable for 128 of 148 donations (by 141 donors) associated with transmission (Figure 1). The 19 donations by the 12 donors linked to multicase clusters occurred in 10 different months. If applicable, the month of donation was approximated by subtracting 16 days (the median age of liquid-stored red blood cells at the time of transfusion; see text) from the transfusion date. The donations linked to the 3 *B. duncani* cases occurred in April (n = 2) and August (n = 1); these data are not included.

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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	新医薬品等の区分	総合機構処理欄
				2011. 10. 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	Simonsen KA, Harwell JI, Lainwala S. Pediatrics. 2011 Oct;128(4):e1019-24. Epub 2011 Sep 2.	公表国	
販売名（企業名）	新鮮凍結血漿-LR「日赤」（日本赤十字社） 新鮮凍結血漿-LR「日赤」成分採血（日本赤十字社）				米国	
研究報告の概要	○早産新生児における輸血関連バベシア症の臨床症状と治療 米国北東部の新生児集中治療室で起きた、新生児輸血関連バベシア症の7症例を報告する。 バベシアに感染していた2つの血液製剤（赤血球）の輸血により7例のバベシア症例が発生した。臨床症状はこの集団において大きなばらつきがあった。超低出生体重児（760g）は最も重症であった。抗生物質での治療は軽度及び無症候性の感染症に効果があった。しかし最も重症であった2症例は長期の多剤処置を伴う2倍量の交換輸血が必要であった。 *Babesia microti*感染症のリスクは現在の血液銀行の業務手順では排除されない。流行地域の新生児科の医師は早産児における輸血関連バベシア症を考慮するべきである。					使用上の注意記載状況・その他参考事項等 新鮮凍結血漿-LR「日赤」 新鮮凍結血漿-LR「日赤」成分採血 血液を介するウイルス、細菌、原虫等の感染 vCJD等の伝播のリスク
	報告企業の意見		今後の対応			
	北米で早産新生児における輸血関連バベシア症が7例発生したとの報告である。		日本赤十字社では問診時にバベシア症の既往を確認し、該当する場合は献血不適としている。今後も引き続き、新規・再興感染症の発生状況等に関する情報の収集に努める。			

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Clinical Presentation and Treatment of Transfusion-Associated Babesiosis in Premature Infants

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KEY WORDS

Babesia, babesiosis/diagnosis, babesiosis/transmission, babesiosis/parasitology, parasitemia/transmission, parasitemia/diagnosis, infant, premature/diseases, infant, premature/parasitology, blood transfusion/adverse effects

ABBREVIATIONS

PCR—polymerase chain reaction
pRBC—packed red blood cell
DOL—day of life
IFA—indirect fluorescent antibody
ELBW—extremely low birth weight

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abstract

We review here 7 cases of neonatal transfusion-associated babesiosis at a NICU in the northeast United States. Transfusion from 2 infected units of blood resulted in the 7 cases described. The clinical presentation was highly variable in this cohort; the extremely low birth weight neonates were the most severely affected. Antibiotic therapy was effective in neonates with mild and asymptomatic infection; however, double-volume exchange blood transfusion with prolonged multidrug treatment was required for the 2 most severe cases. The risk of *Babesia microti* infection is not eliminated through current blood-bank practices. Neonatologists in endemic areas should have a high index of suspicion for babesiosis in premature infants exposed to blood transfusions. *Pediatrics* 2011;128:e1019–e1024

Babesiosis is a zoonotic protozoal illness transmitted primarily by *Ixodes scapularis* ticks in North America. The majority of cases in the Northeast and the upper Midwestern regions are attributed to *Babesia microti*.[1] Transfusion-associated babesiosis is well documented in adults.[2–8] The clinical manifestations range from asymptomatic infection to fulminant disease and death.[9] Babesiosis in neonates occurs through blood transfusion or transplacental transmission.[9–12] Premature infants are at increased risk for babesiosis; they are immunologically compromised and may require multiple blood transfusions during their hospitalization.[13–15] We review here a series of neonatal transfusion-associated babesiosis cases and emphasize the clinical presentation and management strategies for severe disease.

METHODS

These cases occurred in 2 clusters. Index cases were identified clinically and diagnosed by using peripheral blood smears performed for manual white blood cell count and differential. Blood-bank tracing identified other exposed infants. All follow-up testing and quantifications of parasitemia were performed by using thick and thin blood smears. Blood samples for polymerase chain reaction (PCR) were obtained for confirmation of diagnosis on the day of initial positive smear result for the index patients and at the time of initial evaluation for other exposed infants. PCR testing was performed at a single reference laboratory (Mayo Medical Laboratories, Rochester, MN) using licensed methods (Roche Molecular Biochemicals, Indianapolis, IN). Infants in cluster 1 had additional confirmatory testing by PCR and blood smears performed at the Centers for Disease Control and Prevention (Atlanta, GA) using previously reported methods,[16] and all PCR results were concordant. Demographic and transfusion details of all but 1 infant (Table 1) have been included in other reports.[5,11]

Case Reports

Cluster 1

This cluster of cases included 4 very low birth weight (≤1500 g) infants with gestational ages that ranged from 24 to 27 weeks. The index case was a 25-week-gestational-age twin with a birth weight of 760 g. The infant's clinical course was significant for respiratory distress syndrome, chronic lung disease, intraventricular hemorrhage, and anemia of prematurity that required multiple packed red blood cell (pRBC) transfusions. The index transfusion occurred on day of life (DOL) 3.

On DOL 36, the infant's clinical status deteriorated with worsening respiratory status, poor perfusion, hyperthermia, generalized edema, and hepatosplenomegaly. Laboratory tests revealed anemia (hemoglobin: 10.4 g/dL), thrombocytopenia (17 000 cells per μL), and conjugated hyperbilirubinemia (14.4 mg/dL). The infant empirically received ampicillin, gentamicin, and amphotericin B while evaluation for sepsis was performed. All routine bacterial, viral, and fungal study results were negative. A peripheral blood smear performed on DOL 51 revealed that 17% of erythrocytes contained intraerythrocytic parasites consistent with *Babesia* sp. The infant began 20 mg/kg per day of clindamycin intravenously in 3 divided doses and 25 mg/kg per day of quinine orally in 3 divided doses. Double-volume exchange blood transfusion was performed, and the infant's parasitemia level decreased to 3.3%. By day 5 of therapy, parasitemia increased to 5.8% erythrocytes. The infant received a second exchange transfusion, and azithromycin (12 mg/kg per day intravenously) and atovaquone (40 mg/kg per day orally in 2 divided doses) were added to the antimicrobial regimen. The parasite load decreased significantly after the second exchange transfusion; however, low levels of detectable parasitemia persisted. Quinine was discontinued after 8 days of treatment, and all other antibiotics were discontinued on treatment day 28 after 2 peripheral blood smears obtained 3 days apart tested negative for *Babesia* sp (Table 1).

Three infants who received pRBC transfusions from the same donor blood as the index case were identified. Peripheral blood smears from all 3 infants revealed parasites, and the infants were treated with antibiotics (Table 1).[5,11] The peripheral blood smears from the mother and twin sibling of the index patient tested negative for intraerythrocytic parasites. An indirect fluorescent antibody (IFA) test on the plasma obtained from the donor pRBC sample was performed at the Centers for Disease Control and Prevention (CDC), and the results were positive for *B microti* (1:256). However, the results of PCR testing and peripheral blood smears from donor blood performed at the CDC were negative.

Cluster 2

The second cluster of babesiosis cases included 3 low birth weight (≤2500 g) infants who received pRBC transfusions from a single infected donor. The index case was born at 25 weeks' gestational age with a birth weight of 770 g. The infant's clinical course was significant for respiratory distress syndrome, chronic lung disease, intraventricular hemorrhage, and fungal sepsis. The infant received the infected pRBC transfusion on DOL 1. On DOL 33 the infant's clinical status acutely deteriorated with hypotension, respiratory distress, and splenomegaly. Laboratory evaluations revealed anemia (hemoglobin: 7.6 g/dL) and thrombocy-

TABLE 1 Summary of Cases of Transfusion-Associated Babesiosis

Case	Ref No.	Age at Transfusion, d	Gestational Age, wk	Birth Weight, g	Initial Parasitemia[a]	Signs and Symptoms at Time of Diagnosis	Time to Diagnosis, d[b]	Initial *Babesia* PCR Result[a]	Treatment (Duration of Each Antibiotic, d)	Time From First Positive to First Negative Smear Result, d
Cluster 1										
Index case 1	6	2	25	760	+ (17%)	Anemia, thrombocytopenia, hepatosplenomegaly, direct hyperbilirubinemia, respiratory distress	49	+	2 V2EBTs[a]: quinine (8); clindamycin (28); azithromycin (22); atovaquone (22)	22
2		41	24	520	+ (1 organism thick prep)	None	26	–	Clindamycin (9); quinine (9)	1
3	6	1	27	1220	+ (0.9%)	None	51	+	Clindamycin (14); quinine (14)	12
4	6 and 11	57	27	750	+ (1.5%)	Hepatosplenomegaly, anemia, hyperbilirubinemia	47	+	Clindamycin (14); quinine (14)	2
Cluster 2										
Index case 5	6	1	25	770	+ (17.2%)	Hypotension, anemia, thrombocytopenia, splenomegaly, respiratory distress	32	NA	1 V2EBT; clindamycin (16); quinine (6); quinidine (10)	13
6	6	2	27	980	–	Pallor, anemia, thrombocytopenia	35	+	Clindamycin (10); quinine (10)	—
7	6	98	32	1730	–	None	41	+	Clindamycin (10); quinine (10)	—

V2EBT indicates double-volume exchange blood transfusion; NA, not available.
[a] + indicates positive test result; –, negative test result (all PCR tests were performed on the day of first positive smear result or first evaluation for babesiosis).
[b] Days from the day of infected blood transfusion to the day that parasitemia was detected on blood smear or by PCR.

topenia (16 000 cells per μL), and on manual differential *Babesia* sp were suspected. Peripheral blood smear quantification revealed 17% parasitemia. The infant received a double-volume exchange blood transfusion and began clindamycin (30 mg/kg per day intravenously in 3 divided doses) and quinine (25 mg/kg per day orally in 3 divided doses). Five days later, quinine was discontinued and quinidine (0.02 mg/kg per minute intravenously) was started. Antibiotic therapy was discontinued after 3 peripheral blood smears (obtained daily) tested negative for intraerythrocytic *Babesia* sp (Table 1).

Two infants who received pRBC transfusions from the same donor blood were identified. Although blood smears tested negative, the results of PCR tests for *B microti* were positive for both infants. Their management is shown in Table 1. Donor blood from this cluster was not tested.

DISCUSSION

We present a series of transfusion-associated babesiosis cases in infants that encompass a wide spectrum of incubation periods and clinical presentations. We also report use of double-volume exchange blood transfusion as an adjuvant therapy for severe babesiosis in infants. Previous cases of transfusion-associated babesiosis in infants are summarized in Table 2.

Premature infants are at increased risk for transfusion-associated babesiosis. They are more susceptible to infections because of their immature immune systems and functional hyposplenia.[17,18] Also, extremely low birth weight (ELBW) (≤1000 g) infants are more likely to receive frequent transfusions during their first days of life.[13,14] In our report, the 2 most severely affected ELBW infants received infected blood transfusions on DOL 1 and 3. They had complicated neonatal

TABLE 2 Review of Reported Cases of Transfusion-Associated Babesiosis in Infants

Age at Transfusion	Ref No.	Diagnosis	Blood Smear Result	Signs and Symptoms at Time of Diagnosis	Time to Diagnosis	Test Results	Treatment (Duration, d)	Time to Negative Blood Smear Result, d
22 d	2 and 9	Hypoplastic lung	+	Fever	22 d	PCR, +; IFA, +; hamster inoculation, –	Quinine (12); clindamycin (12); atovaquone (8)	8
31 d	2 and 9	Prematurity	+	None	42 d	PCR, +; IFA, +; hamster inoculation, +	Azithromycin (7); atovaquone (7)	4
57 d	2 and 9	Prematurity	–	None	NA	PCR, –; IFA, –; hamster inoculation, –	Azithromycin (7); atovaquone (7)	NA
11 d	2 and 9	Prematurity, necrotizing enterocolitis	–	None	NA	PCR, –; IFA, –; hamster inoculation, –	Observation (NA)[a]	NA
42 d	25	Prematurity	+	Thrombocytopenia, anemia, hyperbilirubinemia, tachypnea, lethargy, jaundice, hepatosplenomegaly	6 wk	IFA, +	Chloroquine (2); clindamycin (7); quinine (7)	7
27 d	26 and 27	Prematurity	+	Apnea, bradycardia, desaturations, splenomegaly, anemia, thrombocytopenia, mildly elevated transaminases levels, respiratory distress	22 d	PCR, +; hamster inoculation, +; IFA, +	Quinine (15); clindamycin (15)	10
49 d	2	Prematurity	+	Respiratory distress, anemia	<40 d	IFA, +; PCR, +	Not available	Parasitemia resolved, day not reported
8 mo	28	Congenital heart disease, status post cardiac surgery	+	Fever, anemia, thrombocytopenia, elevated transaminase levels, pallor, cyanosis, hypoxia	1 mo	PCR, +; IFA, +	Azithromycin (10); atovaquone (10)	5 (parasitemia < 1%)
7 mo	29	Congenital heart disease, status post cardiac surgery	+	Fever, pancytopenia, tachypnea, hepatosplenomegaly, petechiae (eyelids), urinalysis positive for ketones, bilirubin, blood, and protein	25 d	PCR, +	Azithromycin (5); atovaquone (11)	NA

+ indicates positive test result; –, negative test result; NA, not applicable.
[a] Not treated because of necrotizing enterocolitis.

clinical courses that included intraventricular hemorrhage, respiratory distress syndrome, and fungal infection and required prolonged antibiotic therapy and double-volume exchange blood transfusions to resolve the infections. A third ELBW infant received the infected blood on DOL 2 and presented with pallor, anemia, and thrombocytopenia. The incubation period in tick-acquired babesiosis is 1 to 6 weeks; however, in transfusion-associated cases it can be as long as 9 weeks.[19,20] In our series, the incubation period was 5 to 7 weeks. The clinical manifestations of babesiosis range from asymptomatic infection to fulminant disease including acute respiratory failure, congestive heart failure, disseminated intravascular coagulation, liver and renal failure, and splenic infarction.[18] A mortality rate of 5% to 21% has been reported in severe cases.[18] It is important to note that early signs and symptoms of infection were indistinguishable from other causes of sepsis in these premature infants. The diagnosis was made by using peripheral blood smears for the index cases. Exposed infants who were identified by using blood-bank records were considered to be infected if either the peripheral blood smear or PCR testing results were positive.

The recommended treatment for babesiosis includes a combination regimen of clindamycin and quinine or atovaquone and azithromycin for 7 to 10 days.[21,22] In our series, the 3 most severely affected infants were treated for 14, 16, and 28 days, respectively, which demonstrates that for severe illness prolonged treatment may be indicated. Once parasitemia was resolved (on the basis of 2 negative blood smear results obtained 3 days apart [index case 1] or 3 negative blood smear results obtained daily [index 2]), antibiotic therapy was discontinued (Table 1). In both index cases, enteral quinine

was discontinued when the patient remained symptomatic while on therapy. In critically ill premature neonates, enteral absorption through the immature gut may be unreliable, and intravenous quinidine should be considered an alternative. None of the infants in this series had evidence of recurrence of infection before discharge from the NICU.

Exchange transfusion is indicated for severe babesiosis associated with parasitemia of ≥10% or significant organ dysfunction.[18,22] Four adult patients with babesiosis treated with red blood cell exchange transfusion had a 50% to 90% reduction in parasitemia.[23] We report here successful use of exchange transfusion for the treatment of severe babesiosis in 2 ELBW infants who presented with initial parasitemia of 17%. After double-volume exchange blood transfusion, the parasite load in these infants decreased by 83% and 47%, respectively. Babesia parasites survive almost exclusively within erythrocytes; thus, exchange transfusion is beneficial by rapidly reducing the parasite burden and the circulating proinflammatory cytokines.[24]

In endemic areas such as Rhode Island in the northeast United States, an estimated 1 in 21 000 red blood cell units are infected with *Babesia*,[3] which places low birth weight infants at increased risk for transfusion-associated babesiosis. Splitting a single unit of pRBCs for transfusion to multiple neonates led to the clusters of infection described. ELBW infants can develop severe illness when exposed to infected blood in the first few days after birth.

Blood-banking organizations screen blood donors for babesiosis by history of infection alone. Currently, there is no US Food and Drug Administration–licensed screening assay for *B microti* in donated blood products, and no other reliable methods are available for eliminating the risk of transfusion-associated babesiosis. Removal of parasites by leukoreduction and γ irradiation are ineffective, pathogen-removal methods remain experimental, and geographic and seasonal blood-donation deferrals are impractical.[30] The American Association of Blood Banks is actively examining appropriate public health responses for effectively reducing the risk of transfusion-associated babesiosis.[5] Clinical awareness of the range of infections that result from transfusion-associated babesiosis is necessary to facilitate ongoing national discussions and participate in the development of strategies for specifically addressing this important pathogen. At present, selective screening of pRBC units for *Babesia* sp before transfusion to high-risk patients, including low birth weight infants, should be considered in endemic areas.[3]

CONCLUSIONS

Neonatologists, especially those in endemic areas, should consider babesiosis in blood transfusion-exposed infants with unexplained illness and request peripheral blood smear examination for *Babesia* sp. When transfusion-associated babesiosis is diagnosed, prompt notification to the blood bank and testing of all exposed infants is necessary. Review of our and other reported cases (Tables 1 and 2) revealed that 50% to 67% of transfusion-exposed infants develop symptomatic babesiosis. Antibiotic therapy for babesiosis in critically ill neonates may need to be prolonged beyond 7 to 10 days to ensure that parasitemia has resolved. Also, double-volume exchange blood transfusion effectively reduces the parasite load in premature infants.

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医薬品 研究報告 調査報告書

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研究報告の概要

○3例目の輸血感染*Babesia duncani* (*B.duncani*)

背景: 米国で報告されたダニ媒介性及び輸血関連のバベシア症例はほとんどが*Babesia microti* (*B.microti*)に起因し、北東部や中西部で発生している。今回、カルフォルニア在住の59歳、鎌状赤血球症(HbSS)患者で、かつてはWA1型原虫と呼ばれた*B.duncani*による症例を調査した。唯一のリスク因子は赤血球輸血を受けたことであった。

症例報告: この症例は2008年9月に診断された。数カ月にわたり輸血量が増加した後、血液スメアにより赤血球内原虫が発見された。分子及び間接蛍光抗体(IFA)分析により*B.microti*陰性、*B.duncani*陽性(IFA価、1:1024)であった。原虫の完全な18Sリボソームの RNA遺伝子が血液検体から増幅された。DNA配列は1991年に分離された指標WA1型原虫の配列に一致した。すぐに輸血血液の調査が行われ、38人の関連供血者のうち34人が評価されたが*B.microti* IFAが陽性の者はいなかった。カリフォルニア在住67歳の関連供血者の*B.duncani* IFA価が1:4096であり、2009年3月にスナネズミに血液検体を接種し*B.duncani*が分離された。2008年4月の供血から10カ月以上が経っていた。当該患者は2008年5月に関連輸血を受けてから4カ月以上を経て診断された。

結論: この患者は*B.duncani*に起因する輸血関連症例の3例目である。この症例は*B.microti*のための検査では検出できないバベシアが疾病原因となり得ることを強調している。

使用上の注意記載状況・その他参考事項等

新鮮凍結血漿-LR「日赤」
新鮮凍結血漿-LR「日赤」成分採血

血液を介するウイルス、細菌、原虫等の感染
vCJD等の伝播のリスク

9-1

報告企業の意見	今後の対応
輸血に関連した3例目の*Babesia duncani*感染症例の報告である。	日本赤十字社では問診時にバベシア症の既往を確認し、該当する場合は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。

MedDRA/J Ver.14.1J

JRC2012T-001

ORIGINAL ARTICLE

The third described case of transfusion-transmitted *Babesia duncani*

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BACKGROUND: Almost all of the reported US tick-borne and transfusion-associated *Babesia* cases have been caused by *Babesia microti*, which is endemic in the Northeast and upper Midwest. We investigated a case caused by *B. duncani* (formerly, the WA1-type parasite), in a 59-year-old California resident with sickle cell disease (HbSS) whose only risk factor for infection was receipt of red blood cell transfusions.

CASE REPORT: The patient's case was diagnosed in September 2008: intraerythrocytic parasites were noted on a blood smear, after a several-month history of increasing transfusion requirements. Molecular and indirect fluorescent antibody (IFA) analyses were negative for *B. microti* but were positive for *B. duncani* (IFA titer, 1:1024). The complete 18S ribosomal RNA gene of the parasite was amplified from a blood specimen; the DNA sequence was identical to the sequence for the index WA1 parasite isolated in 1991. The patient's case prompted a transfusion investigation: 34 of 38 pertinent blood donors were evaluated, none of whom tested positive by *B. microti* IFA. The implicated donor—a 67-year-old California resident—had a *B. duncani* titer of 1:4096; *B. duncani* also was isolated by inoculating jirds (Mongolian gerbils) with a blood specimen from March 2009, more than 10 months after his index donation in April 2008. The patient's case was diagnosed more than 4 months after the implicated transfusion in May 2008.

CONCLUSIONS: This patient had the third documented transfusion case caused by *B. duncani*. His case underscores the fact that babesiosis can be caused by agents not detected by molecular or serologic analyses for *B. microti*.

Babesiosis is a tick-borne disease caused by intraerythrocytic parasites that also are transmissible by transfusion.[1-8] During the past three decades (1979-2009), more than 150 US cases of transfusion-associated babesiosis have been recognized,[1,2] most of which have been linked to red blood cell (RBC) components (liquid stored or frozen deglycerolized[9]); whole blood–derived platelets (PLTs) also have been implicated, presumably because of residual RBCs or extracellular parasites in PLT concentrates.[2,4,10] No test has been approved by the Food and Drug Administration (FDA) for

ABBREVIATIONS: ICU = intensive care unit; IFA = indirect fluorescent antibody.

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The findings and conclusions in this report are those of the authors and do not necessarily represent the policy of the Centers for Disease Control and Prevention.

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TRANSFUSION **;**:**–**;**.

screening US blood donors for evidence of *Babesia* infection.[2,3] Prevention of transfusion transmission currently relies on indefinite deferral of potential donors who have a history of babesiosis (those who answer "yes" to the question "have you ever had babesiosis?") and temporary deferral of donors who are febrile or anemic. However, persons who are otherwise healthy can meet the criteria for donating blood despite being infected; low-level parasitemia can persist for weeks to months, sometimes longer than a year.[1-3,8] *Babesia* infection also can be severe, even life-threatening,[1-4,9,11,12] particularly in persons who are asplenic, at either extreme of age, or immunocompromised, which are common characteristics of transfusion recipients.

Babesia microti, which is endemic in parts of the Northeast and upper Midwest, accounts for almost all of the reported US *Babesia* cases, including some transfusion cases documented in other US regions in the context of movement of donors or blood components.[2,6,9,11-14] The WA1- (for "Washington 1") type parasite, which was named *B. duncani* in 2006,[15] also has been associated with both routes of transmission, although its tick vector has not yet been identified. The index WA1 case occurred in 1991 in Washington State, in an immunocompetent 41-year-old man, who had a presumptive tick-borne case.[16] The next described case caused by the parasite now called *B. duncani* was transfusion associated: it occurred in 1994 in Washington, in a 76-year-old patient with myelodysplasia who underwent cardiac surgery.[17] The second documented transfusion case occurred in 2000 in California, in a preterm infant.[18] Both of these transfusion cases were linked to RBC components from healthy, 30-some-year-old donors who had presumptive tick-borne cases. Here we describe the third identified *B. duncani* transfusion case, which was diagnosed in 2008 in California, in a 59-year-old man with a transfusion-dependent hemoglobinopathy who had become refractory to RBC transfusions.

MATERIALS AND METHODS

The Centers for Disease Control and Prevention (CDC) conducted reference laboratory testing for *Babesia* infection. Giemsa-stained thin blood smears were examined by light microscopy for intraerythrocytic parasites; *B. duncani* is morphologically indistinguishable from *B. microti*.[15] Serum and plasma specimens were tested in serial fourfold dilutions, with indirect fluorescent antibody (IFA) assays for reactivity to *B. duncani* and *B. microti* antigens.[16,19]

Whole blood specimens were analyzed by molecular techniques. For *B. microti*, two-step nested polymerase chain reaction (PCR) was conducted.[8,20] The molecular approach used for *B. duncani* has been described previously, including the methods for DNA extraction, amplification, and sequencing.[21] In brief, the complete 18S ribosomal RNA (rRNA) gene was amplified by PCR, with primers that amplify DNA from parasites in the order Piroplasmida: the PCR primers were the forward primer CRYPTOFL (5'-AACCTGGTTG ATCCTGCCAG TAGTCAT-3') and the reverse primer CRYPTORN (5'-GAATGATCCT TCCGCAGGTT CACCTAC-3'). For the organism referred to as the recipient's parasite, both strands of the PCR product were sequenced (BAB1615; GenBank Accession Number HQ289870); the sequence of the 18S rRNA coding region was compared with the sequence CDC obtained for the WA1 parasite isolated from the index case patient in 1991[18] (BAB2; Accession Number HQ285838).

Whole blood specimens (up to 1 mL) were inoculated intraperitoneally into jirds (Mongolian gerbils; *Meriones unguiculatus*), which are competent hosts for *B. duncani*.[16] The jirds were monitored weekly, up to 8 to 10 weeks or until positive, by examination of Giemsa-stained smears of blood obtained by tail snip. Animal use protocols were approved by CDC's Institutional Animal Care and Use Committee.

CASE REPORT

In late September 2008, a case of *B. duncani* infection was diagnosed in a 59-year-old California resident who had sickle cell disease, an autoinfarcted spleen, a 10-year history of transfusion-dependent anemia, and a several-month history of deteriorating health and increasing transfusion requirements. At baseline, RBC exchange transfusions at 3- to 4-week intervals sufficed to maintain a hemoglobin (Hb) level of 10 to 11 g/dL. Comorbidities included congestive heart failure and chronic renal insufficiency (baseline creatinine level of approx. 3 mg/dL), both of which had been controlled with medical therapy.

In early June 2008, several months before babesiosis was diagnosed, he was evaluated because of a febrile illness. He had a 1-week history of symptoms, which included anorexia, nausea, diarrhea, respiratory congestion, stiff neck, and 3 days of fever (37.2-38.9°C); his Hb level was 7.1 g/dL. The diagnoses included otitis media, viral infection, and dehydration; amoxicillin-clavulanate and fluids were prescribed.

During the summer of 2008, he remained afebrile. However, he had frequent outpatient and inpatient evaluations because of weakness, fatigue, and shortness of breath, which were attributed to anemia. In mid-July, his Hb level was 8.5 g/dL. After an exchange transfusion 1 week later, the interval between transfusions became progressively shorter than his 3- to 4-week norm. In mid-August, he noted darkening of his urine. He became refractory to transfusions, with Hb levels of not more than 6 g/dL, despite twice-weekly therapy with epoetin alfa. In addition, he received corticosteroid therapy from late August through mid-September for warm-antibody autoimmune hemolytic anemia, which compounded his transfusion requirement but was considered insufficient to account for the severity of his anemia. The corticosteroid regimen included a 40-mg dose of methylprednisolone (accompanied by one dose of intravenous immune globulin), followed by tapering doses of prednisone. The patient received another 40-mg bolus of methylprednisolone in early September, with tapering doses of methylprednisolone thereafter. A Hb level of 6.9 g/dL in late August, after corticosteroid therapy had been initiated, prompted hospital admission, including a 10-day stay in the intensive care unit (ICU). On admission to the ICU, his Hb level was 4.9 g/dL. Diagnostic considerations included thrombotic thrombocytopenic purpura and a delayed-type transfusion reaction. Because of acute (superimposed on chronic) renal failure, hemodialysis was initiated and was continued thereafter.

He returned to the ICU later in September, with a 1-week history of nausea, vomiting, loose stools, anorexia, weakness, and pain (in the lower back and knees), in addition to persistent fatigue and weakness. On examination, his temperature was 37.3°C, his blood pressure was 76/45 mmHg, he was icteric and somnolent but oriented, and he had tremors in his tongue and upper extremities. Laboratory values included a Hb level of 5.8 g/dL, PLT count of 135×10^9/L, white blood cell count of 15.3×10^9/L (45% neutrophils, 20% lymphocytes, and 36% monocytes), reticulocyte count of 16.5%, total bilirubin of 9.6 mg/dL, aspartate aminotransferase of 464 U/L, and alanine aminotransferase of 117 U/L.

In late September, babesiosis was diagnosed, when intraerythrocytic parasites, including pathognomonic tetrads ("Maltese-Cross" forms), were noted on a blood smear (Fig. 1); according to the hospital laboratory, approximately 12% of the RBCs were infected. During retrospective examination of available smears, rare parasites were noted on a smear from mid-July 2008; none were found on a smear from November 2007. No smears between November and July were available.

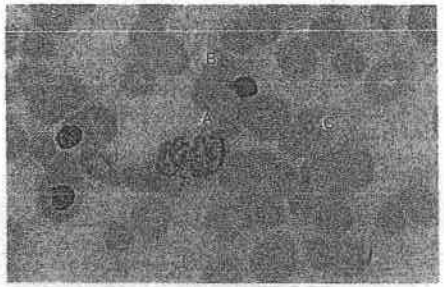

Fig. 1. Photograph of a Wright-Giemsa–stained peripheral smear of blood obtained from the patient in September 2008, showing typical *Babesia* forms: (A) a tetrad ("Maltese-Cross" dividing merozoite); (B) a piriform (tear drop); and (C) a ring-like trophozoite (magnification ×1000).

After babesiosis was diagnosed, combination therapy with clindamycin (600 mg every 6 hr by intravenous infusion) and quinine sulfate (648 mg loading dose per nasogastric tube, followed by 324 mg every 12 hr) was initiated. In addition to a several-week course of antimicrobial therapy, he received an 8-unit RBC exchange transfusion. Parasites were not demonstrable on several follow-up blood smears in October; his transfusion requirement also decreased. Because of persistent weakness and pain, which were attributed to polyneuropathy of critical illness, he was transferred to a rehabilitation facility. Over the next 6 months, his neuropathy and mentation gradually improved; he continued to receive hemodialysis.

Babesia laboratory testing

After a commercial laboratory obtained negative serologic results for *B. microti*, the CDC was consulted and conducted reference laboratory testing. CDC confirmed the diagnosis of babesiosis and determined that the etiologic agent was *B. duncani*. Results of molecular and IFA analyses of a blood specimen from late September 2008 were negative for *B. microti* but were positive by both modalities for *B. duncani* (IFA titer, 1:1024). PCR amplification of the complete 18S rRNA gene yielded a specific product of approximately 1700 bp. DNA sequencing analysis showed that the gene was 1768 bases long. The DNA sequence for the recipient's *Babesia* parasite was identical to the sequence for the index WA1 parasite that was isolated in 1991 and analyzed at CDC (see Materials and Methods for GenBank accession numbers). The CDC's 18S rRNA sequence data differ slightly (approx. 0.2%) from another laboratory's sequence for the index WA1 isolate (GenBank Accession Number AF158700), which may reflect different methods for sequencing and sequence editing. The recipient's *Babesia* parasite also was isolated by inoculating jirds with a blood specimen from the patient.

Transfusion investigation

The patient's only risk factor for *Babesia* infection was receipt of RBC transfusions: he lived in an urban area of a northern California county and did not have a history of rural outdoor activities during the previous 2 years. Diagnosis of his case of babesiosis prompted a multiagency investigation that encompassed the transfusions he received during September 2007 through June 2008, all of which were of leukoreduced, nonirradiated RBCs. Among 38 pertinent donors from the American Red Cross Northern California Region (Oakland, CA) and the Blood

Centers of the Pacific (San Francisco, CA), 34 provided specimens for testing; no segments from the original units were available.

None of the 34 tested donors had detectable antibodies to *B. microti*. Only one donor—Donor A (the implicated donor)—tested positive by *B. duncani* IFA (see below). The RBCs from Donor A were collected in April 2008, when his Hb value was 15.2 g/dL. The RBCs were transfused to the patient 25 days later, in May. The interval from this transfusion to the patient's diagnosis of babesiosis (in late September) was 130 days, and the interval from transfusion to the patient's first known positive smear (in mid-July) was 56 days. The case patient was the only recipient of a cellular component from the April donation.

Two specimens from Donor A were collected in November 2008 and March 2009 for *Babesia* testing. The *B. duncani* IFA titer was 1:4096 for both specimens, which were tested in parallel, on the same day. Molecular analyses and blood smears were negative. However, he had protracted, parasitologically confirmed infection: aliquots from the March 2009 specimen (>10 months after the index donation) were inoculated into two jirds, both of which were demonstrably parasitemic when examined on Day 21 postinoculation.

Donor A was a healthy 67-year-old resident of the San Francisco Bay Area, who was an avid hiker and mountain biker, including in the Bay Area, elsewhere in California (e.g., in the central Sierra Nevada foothills and in multiple regional and national parks), and in the Northwest. His interstate travel included hiking trips in Washington and British Columbia (the fall of 2004) and in Wyoming, Montana, and Idaho (the fall of 2007). Although he did not recall any tick bites, he reported having a possible tick bite reaction on his right shoulder after a hike in the rural Bay Area in April 2008, the month of the index blood donation. He recalled having a mild flu-like illness in October 2007, after a hike in the southern Bay Area. Although his wife had not accompanied him on that hike, she developed a similar illness; a blood specimen she provided in May 2009 did not react to *B. duncani* antigens in IFA testing at CDC.

Since 2007, Donor A had donated blood five times; the index donation in April 2008 was the third in the series of five. The two subsequent donations included one in early August 2008 (the recipient was lost to follow-up) and one in late September (the blood was discarded when the transfusion investigation was initiated). After Donor A was implicated, he was indefinitely deferred from donating blood. His two previous donations were in November 2007 (the blood was discarded during processing because of incomplete filtration) and in January 2008. RBCs from his January 2008 donation were transfused to an oncology patient in northern California, who reportedly was asymptomatic when evaluated in March of the following year. In April 2009 (415 days posttransfusion), a blood specimen from the recipient was collected for *Babesia* testing: the *B. duncani* IFA titer was 1:256, molecular analyses and a blood smear were negative, and two jirds inoculated with 22-day-old blood did not develop demonstrable parasitemia.

DISCUSSION

We investigated a case of *B. duncani* infection that was diagnosed in late September 2008, in a chronically transfused patient who had become refractory to transfusions. The patient had three reasons for hemolytic anemia: sickle cell disease, which previously had been well controlled; warm-antibody autoimmune hemolytic anemia, which was diagnosed in August 2008; and *B. duncani* infection, which was diagnosed more than 4 months after the May 2008 transfusion that was implicated in the multiagency investigation of the RBC transfusions and donors since September 2007. In retrospect, rare parasites were found on an extant blood smear from mid-July 2008, approximately 2 months after the implicated May transfusion. The interval from the implicated transfusion to onset of clinical manifestations is unclear. However, if the patient's only documented febrile illness, which occurred in early June 2008, was caused by babesiosis, the incubation period was approximately 2 weeks. His *Babesia* infection responded to therapy with clindamycin plus quinine, which remains the standard of care for severely ill patients.[1] However, his clinical course was complicated by prolonged morbidity from multifactorial renal failure and polyneuropathy.

Both the patient and the implicated donor (Donor A) were residents of northern California, were seropositive by *B. duncani* IFA (but seronegative by *B. microti* IFA), and had parasitologically confirmed infection. Donor A, who was otherwise healthy, had no overt manifestations of *Babesia* infection even though he was 67 years old (8 years older than the recipient). Although the duration of his infection is not known, his case underscores that *B. duncani*, like *B. microti*, can be associated with protracted, asymptomatic parasitemia: *B. duncani* was isolated from a blood specimen collected in March 2009, more than 10 months after his index donation in April 2008. Similarly, for the first documented *B. duncani* transfusion case, the parasite was isolated from a specimen obtained from the donor in March 1995, 7 months after the index donation in August 1994.[17] Although limited data suggest that *B. duncani* may be more pathogenic than *B. microti* in experimentally inoculated mice and hamsters,[15] the potential relevance of these animal data to human infection is unknown.

For Donor A, the possibility that recipients of cellular components from other donations became infected could not be proven or excluded. Although Donor A undoubtedly was infected when he donated again in August 2008, no information was available about the recipient. Donor A's infection status back in January 2008 is unknown, and the *Babesia* test results more than 13 months posttransfusion for the recipient of RBCs from that donation (an oncology patient) are noteworthy but not definitive—in particular, the patient's *B. duncani* IFA titer of 1:256. The threshold for considering a *B. duncani* IFA result positive has not been well established: to our knowledge, fewer than 10 *B. duncani* cases have been parasitologically confirmed and monitored serologically. The oncology patient's possible case of *B. duncani* infection was not parasitologically confirmed, only one specimen was tested, and limited epidemiologic and clinical information was available. However, the negative results of PCR analyses and animal inoculation do not exclude the possibilities that the patient either had been or still was infected, regardless of the mode of transmission; these methods are not sufficiently sensitive for reliable detection of low-level parasitemia.

Much remains unknown about *B. duncani*, such as the interrelated issues of its geographic distribution, tick vector, and reservoir host(s),[15] as well as the incidence and prevalence of infection in humans, including blood donors and recipients. The case we described, the third documented transfusion case caused by *B. duncani*, underscores that the difficulties inherent to detecting and investigating *Babesia* cases are compounded in patients who have been chronically transfused, have multicause hemolytic anemia, and are infected with species other than *B. microti*. Human infection with *B. duncani* and other novel *Babesia* agents[21] is not detected by serologic or molecular assays for *B. microti*, which has ramifications not only for diagnostic testing and transfusion investigations but also for potential future screening of blood donors.[2] Effective measures for preventing transfusion transmission of *Babesia* parasites are needed. Although the highest near-term priority is the development and/or implementation of FDA-approved donor-screening test(s) for *B. microti*, there also is a need for *Babesia* genus- (vs. species-) level assays.

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CONFLICT OF INTEREST

None.

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別紙(2)-2

別紙様式第２

医薬品　研究報告　調査報告書

識別番号・報告回数		報告日	第一報入手日 2012年2月14日	新医薬品等の区分 該当なし	厚生労働省処理欄
一般的名称	①②フィブリノゲン加第XIII因子 ③④人血液凝固第XIII因子	研究報告の公表状況	Deconstructing the risk for malaria in United States donors deferred for travel to Mexico Transfusion　2011:51(11)2398-2410	公表国 米国	
販売名（企業名）	①ベリプラストＰ コンビセット ②ベリプラストＰコンビセット組織接着用 ③フィブロガミンＰ ④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

【背景】米国においてメキシコ旅行はマラリアによる供血延期の最も大きな割合を占めるが、メキシコ国内の大半の地域でマラリア感染リスクは極めて低く、輸血の安全性と有効性のバランスが不均衡であることが示唆される。本研究では、メキシコの一部地域で血液ドナーの供血延期要件を緩和すべきか検証した。【方法】2006年に血液センター6施設において、メキシコに旅行したため供血を延期された血液ドナー計885名を対象とし、ドナーの感染リスクを滞在先の現地住民のマラリア感染率から算出し、滞在期間により調整した。血液センターの供血延期数の比率をメキシコ旅行により供血延期されている推定66,554名/年に応用することで、米国全体における影響を推測した。【結果】メキシコにおけるマラリア感染は2000年に16州234郡で7,272例発生していたが、2009年では人口比3％以下の10州115郡で2,595例と減少していた。また、感染リスクは地域によって異なり、Chiapas州およびOaxaca州が全症例数の75％を占めていた。72％のドナー（推定47,939名/年）がユカタン半島にあるQuintana Roo州を旅行したことで供血を延期されているが、ドナーの感染リスクは0.0080例/年または125年に1例であった。感染リスクの高いOaxaca州に旅行して供血を延期された1,847名を除けば、残りの64,707名におけるマラリア感染リスクは20年に1例であった。【結論】Quintana Roo州のようなマラリア感染リスクが極めて低いと判断されるメキシコ国内の地域に関しては、供血延期要件を緩和すべきである。

使用上の注意記載状況・その他参考事項等

10

報告企業の意見	今後の対応
マラリアは赤血球内にマラリ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。

Deconstructing the risk for malaria in United States donors deferred for travel to Mexico

Bryan Spencer, Steven Kleinman, Brian Custer, Ritchard Cable, Susan L. Wilkinson, Whitney Steele, Patrick M. High, and David Wright for the NHLBI Retrovirus Epidemiology Donor Study-II (REDS-II)

BACKGROUND: More than 66,000 blood donors are deferred annually in the United States due to travel to malaria-endemic areas of Mexico. Mexico accounts for the largest share of malaria travel deferrals, yet it has extremely low risk for malaria transmission throughout most of its national territory, suggesting a suboptimal balance between blood safety and availability. This study sought to determine whether donor deferral requirements might be relaxed for parts of Mexico without compromising blood safety.

STUDY DESIGN AND METHODS: Travel destination was recorded from a representative sample of presenting blood donors deferred for malaria travel from six blood centers during 2006. We imputed to these donors reporting Mexican travel a risk for acquiring malaria equivalent to Mexican residents in the destination location, adjusted for length of stay. We extrapolated these results to the overall US blood donor population.

RESULTS: Risk for malaria in Mexico varies significantly across endemic areas and is greatest in areas infrequently visited by study donors. More than 70% of blood donor deferrals were triggered by travel to the state of Quintana Roo on the Yucatán Peninsula, an area of very low malaria transmission. Eliminating the travel deferral requirement for all areas except the state of Oaxaca might result in the recovery of almost 65,000 blood donors annually at risk of approximately one contaminated unit collected every 20 years.

CONCLUSION: Deferral requirements should be relaxed for presenting donors who traveled to areas within Mexico that confer exceptionally small risks for malaria, such as Quintana Roo.

Transfusion-transmitted malaria (TTM) is a potentially lethal outcome of blood transfusion, but one that is uncommon in the United States. On average, the past two decades have seen less than one case of TTM per year, representing a rate of less than 0.1 per 10^6 red blood cell transfusions.[1-3] TTM prevention currently relies on exclusion of donors who might present risk for malaria infection. Based on requirements and recommendations of the Food and Drug Administration (FDA)[4] and the AABB,[5] the deferral period is 3 years for those who report a history of malaria infection or of prior residence in a malaria-endemic country as defined by the Centers for Disease Control and Prevention (CDC),[6] whereas it is 1 year for donors with travel to parts of countries considered endemic for malaria by CDC.

The number of presenting or productive donors with nonzero risk for harboring malaria parasites is essentially unknown, as is the relative likelihood for infection across the different malaria deferral categories. However, empirical evidence in the United States[1,2,7] and other countries[8-11] clearly implicates donors from sub-Saharan Africa in cases of TTM over the past three decades. While many of these donors were improperly accepted due to an error during health history screening, others were rare biologic outliers whose semi-immune status allowed for *Plasmodium* parasite carriage for several years. This occurred most often with *Plasmodium falciparum* and also with *P. malariae*, a more benign species known to remain undetected for decades.[12]

In contrast to those with long-term residence in sub-Saharan Africa and a few other highly endemic areas, donors reporting routine travel to malarial areas appear to confer relatively low risk for transmitting malaria to recipients of blood products. In fact, over a 28-year period, only one donor out of 32 implicated in TTM cases was a native-born US resident on routine travel.[1,2,7] While denominator data that would allow for accurate risk comparisons across the different deferral categories are lacking, there can be no doubt that the 1-year deferral period for short-term travelers has a significant impact on blood availability. A recent analysis by our consortium estimates that annual deferrals by US blood centers might surpass 150,000,[13] and unpublished data suggest that several times that many might self-defer.[14]

To assess the risk that donors who report a travel history requiring malaria deferral might actually be infected with malaria parasites, our prior study detailed the travel destinations of a representative sample of blood donors with malaria travel deferral.[13] Using public data sources on imported malaria in the United States[15-20] and on numbers of travelers to different countries and regions,[21,22] we developed region-specific risks for malaria infection that suggested a 1000-fold greater risk for travelers to Africa versus Mexico. Traveler risk to Mexico was estimated as roughly 1 in 800,000 for those who visited an area triggering malaria deferral. We concluded that these data supported the relaxation or elimination of the year-long deferral period for travel to Mexico, with a negligible marginal increase in risk balanced by the recovery of tens of thousands of donors annually.[13]

Given the large number of deferrals after travel to Mexico, and the long-term trends in malaria incidence there, this article reports a follow-up analysis focusing only on this country. Mexico is a country of low and declining risk for malaria, one where the remaining areas of natural transmission are characterized as scattered foci in rural areas populated by indigenous inhabitants.[23] Although firm data are unavailable on how many US tourists visit any given location within Mexico, US malaria surveillance figures support the interpretation that most travelers visit areas with very low risk for malaria. For each year from 2000 to 2007, an average of approximately six and a maximum of 11 US residents have acquired malaria in Mexico that was diagnosed following return to the United States,[7,15-20,24] out of an average of roughly 20 million visitors annually with at least an overnight stay.[22] Furthermore, a disproportionate share of these malaria cases occurred in Mexican citizens now residing in the United States,[25] so the risk for routine, US-born travelers appears exquisitely low.

To develop risk estimates for malaria infection in blood donors that are independent of the travel destination within Mexico for overall US travelers, this article adopts an alternate method that derives donor risk from malaria risk figures for the local populations of the areas visited. Specifically, we recorded destination(s) of travel within Mexico for the same cohort of deferred donors described in the earlier analysis[13] and used malaria surveillance indicators developed by the Mexican Ministry of Health for the same areas during the same time frame as a measure of potential malaria risk faced by blood donors deferred for travel to Mexico. Because the Mexican morbidity data reflect risk for year-round residents, we adjust the donor risks for duration of exposure. To provide a basis for selective alterations to current deferral requirements for Mexico, we disaggregate the results by state, since distribution of risk across the country varies significantly.

MATERIALS AND METHODS

Source of data on presenting US blood donors deferred for travel to malaria-endemic areas in Mexico

Six blood centers participating in the Retrovirus Epidemiology Donor Study-II (REDS-II) program sponsored by the National Heart, Lung, and Blood Institute (NHLBI) provided data for this analysis. These centers represent geographically and demographically diverse populations and collectively account for more than 8% of annual allogeneic blood collections in the United States.[3] The REDS-II blood centers include the Blood Centers of the Pacific (BCP; San Francisco, CA); BloodCenter of Wisconsin (BCW; Milwaukee, WI); Hoxworth Blood Center, University of Cincinnati (HOX; Cincinnati, OH); the Institute for Transfusion Medicine (ITxM; Pittsburgh, PA); the American Red Cross, New England Region (NEARC; Dedham, MA); and the American Red Cross, Southern Region (SARC; Douglasville, GA). Each center retrieved blood donation records from the first 60 donors deferred for malaria travel in either the even-numbered (BCW, ITxM, SARC) or odd-numbered months (BCP, HOX, NEARC) throughout 2006 to ensure that seasonal patterns in travel were captured. Data were recorded on donor demographics, date of presentation, dates of travel in malaria-endemic regions, and the destination country or countries with malaria risk for up to five countries.

ABBREVIATIONS: API = annual parasitological index, number of cases reported per 1000 residents per year; BCP = Blood Centers of the Pacific; BCW = BloodCenter of Wisconsin; HOX = Hoxworth Blood Center, University of Cincinnati; ITxM = Institute for Transfusion Medicine; NEARC = American Red Cross, New England Region; SARC = American Red Cross, Southern Region; TTM = transfusion-transmitted malaria.

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Countries were ranked by malaria risk,[26,27] and specific locations triggering malaria deferral were recorded verbatim from the blood donation records for the two countries with highest risk for malaria. This analysis reports only on the subset of donors for whom Mexico either was the only country visited with malaria risk or was the country with the highest risk. Deferral records lacking the destination country or the date that deferral began were excluded from the analysis.

Estimate of annual deferrals of US blood donors for malaria-risk travel to Mexico

The proportion of malaria travel deferrals that were allocated to Mexico for the six REDS-II centers was used to estimate the annual number of malaria travel deferrals associated with Mexico for US donors overall. Annual data on allogeneic donations and malaria travel deferrals were recorded and summed across all centers, and each center's contribution to national estimates was weighted by its share of the collective REDS-II malaria travel deferrals. Based on their aggregate contribution of 8.07% of US allogeneic donations,[3] the blood centers' estimates for malaria deferrals were multiplied by 12.4 to extrapolate to the US overall. These figures have been updated from the earlier article[13] to account for the availability of US donation data from 2006,[3] which increases the projections of US deferrals for malaria travel from 150,537 to 161,105. When a donor reported more than one trip with a visit to a malaria-endemic area, the most recent trip was chosen for analysis. When more than one geographic region within Mexico was visited, the donor's visit was allocated to the area of higher risk for malaria infection, based on Mexican public health data. Donor's travel destination was assigned to the smaller administrative unit possible between state- and county-level destinations. Donors for whom location within Mexico was not available were assumed to represent randomly missing data and were accounted for by proportional weighting for those donors who did report location, for each of the six blood centers.

Criteria for malaria travel deferral to different areas of Mexico

For US travelers, risk for malaria infection is described by the CDC on a country-by-country basis,[6] and by FDA requirement this information is used as the basis on which donor acceptability is determined.[4] For the time frame of this study–donor presentation during 2006 and donor travel during 2005 through 2006—the description of malaria risk in Mexico was as follows:

> Risk in rural areas, including resorts in rural areas of the following states: Campeche, Chiapas, Guerrero, Michoacán, Nayarit, Oaxaca, Quintana Roo, Sinaloa, and Tabasco. In addition, risk exists in the state of Jalisco (in its mountainous northern area only). Risk also exists in an area between 24° N and 28° N latitude and 106° W and 110° W longitude, which lies in parts of Sonora, Chihuahua, and Durango. No malaria risk exists along the United States-Mexico border. No malaria risk exists in the major resorts along the Pacific and Gulf coasts.[6]

The specific job aids or work instructions provided for health historians of each REDS-II blood center might have differed in detail during 2006, but the referent for each center would have been the foregoing text from CDC. Figure 1 shows a map of Mexico,[28] with those states named in the above excerpt shaded in gray. Depending on the specificity of information provided by donors with travel to Mexico, as well as on the detail of information available in reference materials for health historians, travel to one of the shaded states during 2005 through 2006 might not have necessarily triggered a deferral. It would, however, at a minimum require consultation with support documentation to exclude travel to a malaria risk area for the donor to be judged acceptable to donate.

Estimated risk for malaria infection in US travelers to different areas of Mexico

Population-adjusted risk measures for year-round Mexican residents at the state level are available from eight endemic states which together account for 98.5% of reported cases in 2005.[29] County-level risk measures were derived from malaria case counts at the county level for 2005[30] and the 2005 census population figures for each county.[31] State- and county-level risk measures for year-round Mexican residents were used as a proxy for potential malaria risk which US residents might face during travel to the same areas, appropriately adjusted for duration of exposure. Donors eligible for inclusion in this study—those deferred during calendar year 2006—reported travel that occurred during both 2005 and 2006. Mexican surveillance data from 2005 only are used to develop risk estimates due to unavailability of 2006 county-level malaria surveillance data. To evaluate longitudinal trends in malaria incidence in Mexico, and to establish any significant changes in the amount or distribution of malaria subsequent to the period used for our study, we referenced several years of publicly available Mexican government surveillance data.[32]

Duration of exposure was estimated by calculating donor-reported dates for beginning and ending of exposure in risk areas. This measure was available for donors from three of the six blood centers, and it was not possible to distinguish donors who simply reported the duration of their entire trip versus the proportion who correctly provided dates only for the portion of travel subject to deferral

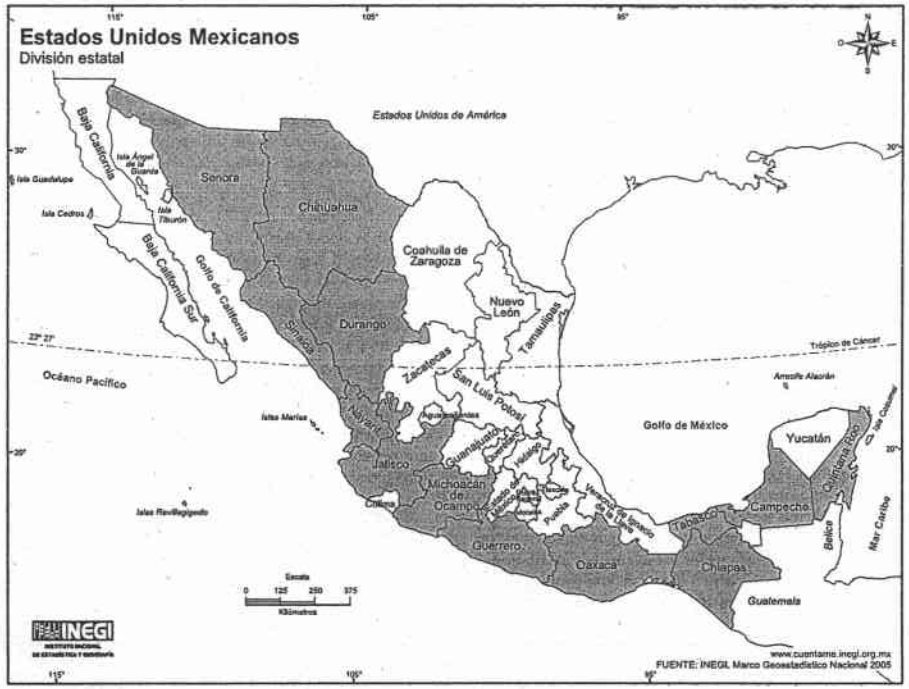

Fig. 1. States in Mexico where US CDC reports risk for malaria, 2005 through 2006. Map adapted from Instituto Nacional de Estatística e Geografía.[28]

due to presumed malaria risk. After reviewing the distribution of results, we chose a uniform duration of exposure (7 days) that encompasses over 70% of those donors with both start and end dates recorded.

Transfusion risk from blood donors with deferrable travel to Mexico

Transfusion risk is defined in this article as the risk that a donor might be accepted for donation while asymptomatically parasitemic, which we assume is equivalent to the risk that a donor might become infected with malaria while on travel in Mexico. Our risk estimates assume the absence of any deferral period for travel to Mexico, which means the donor could be found acceptable to donate the very day upon return from travel. Because the risk for asymptomatic malaria infection diminishes with increasing interval between return from travel and date of presentation—approximately 50% of *P. vivax* infections manifest within the first month and 70% within the first 3 months[13]—the actual risk from abolishing the deferral for Mexico might be even lower than we estimate.

RESULTS

Malaria travel deferrals to Mexico for REDS-II donors and extrapolation to the United States annually

The six REDS-II centers reported a total of 13,007 deferrals for travel to malaria-endemic areas by US residents in 2006 (Table 1), of which 2160 were sampled for further analysis. The required data were available for 2108 of these (97.6%), of which 885 donors were deferred for malaria travel to Mexico. Based on weighting that is proportional to each blood center's malaria deferral count, extrapolating to the US yields an estimated 66,554 deferrals annually for malaria travel to Mexico. The change from 870 donors deferred for travel to Mexico in the earlier analysis[13]

TABLE 1. Malaria travel deferrals to Mexico at six REDS-II Blood Centers, 2006

	BCP	BCW	HOX	ITxM	NEARC	SARC	Total	Weighted extrapolation to United States
Number of travel deferrals	2761	2128	1122	1622	3570	1804	13,007	161,105
Number of travel deferrals sampled	334	353	356	359	360	346	2,108	
Deferrals to Mexico	123	230	159	119	130	125	885	66,554
Travel deferrals due to travel to Mexico (%)	36.8	65.2	44.7	33.1	36.1	35.8	42.0	41.3
Mexico deferrals with identifiable location (%)	62	98	99	92	95	52	85	

TABLE 2. Mexican population at risk and reported malaria cases at the state level, 2005

State	Population, 2005	Population at risk, 2005	Number of counties reporting cases, 2005	Malaria cases, 2005	Population-adjusted risk, API (and 95% CI*), for malaria, 2005
Durango	1,554,948	14,126	3 of 39	114	8.07 (6.71-9.70)
Oaxaca	3,716,837	193,669	19 of 570	1432	7.39 (7.02-7.79)
Chihuahua	3,432,518	33,126	8 of 67	181	5.46 (4.72-6.32)
Sonora	2,487,066	5,777	4 of 72	29	5.02 (3.49-7.22)
Tabasco	2,069,522	119,497	9 of 17	97	0.81 (0.67-0.99)
Chiapas	4,417,084	1,062,455	65 of 119	852	0.80 (0.75-0.86)
Sinaloa	2,771,148	559,254	10 of 18	208	0.37 (0.32-0.43)
Quintana Roo	1,091,496	127,808	2 of 8	11	0.09 (0.05-0.16)
Nayarit and other states	24,466,964	684,288	13 of 667	42	0.06 (0.04-0.08)
Total	46,007,583	2,800,000	133 of 1,577	2966	1.06 (1.02-1.10)

* CIs do not account for temporal or geographic variation, and hence inference is to only the year 2005 and is specific to each state.

derives from changing from a macroregional to a country-based analysis, for which Mexico was the country of higher risk.

Risk for malaria infection in US travelers to Mexico

Population-adjusted risks for malaria at the state level are shown in Table 2, supporting the characterization of risk areas within Mexico as mostly scattered foci rather than large swaths of the national landscape and demonstrating that most residents of endemic areas face very low risk for malarial illness. First, of the 2.8 million Mexican residents living in risk areas, fewer than 10% reside in areas with an annual risk for malaria greater than 1 per 1000 (an annual parasitological index [API] of 1 means one case is reported per 1000 residents). Even in these regions—Durango, Chihuahua, and Sonora in the Northwest and Oaxaca in the South—the population at risk represents a small proportion of each state's population, and the number of counties with cases is low compared to each state's overall number of counties. Although the risk faced by residents of these four states is large relative to residents of the other endemic states, this elevated risk is confined to a very small fraction of the population. For Quintana Roo and four other states, which collectively account for 53 reported cases, the risk is less than 1 per 10,000 residents per annum.

Table 3 contextualizes the malaria risks across states in 2005 within a long-term trend of increasingly lower malaria risk in fewer and more circumscribed geographic areas. Compared to 1985, when more than 133,000 cases were reported from all but two Mexican states,[33] the number of cases declined by 95% by the year 2000, and within the past decade has been reduced by an additional 60%. Active transmission of *Plasmodium* parasites is now limited to 115 counties distributed across 10 states, down from 234 counties in 16 states in 2000, and applies to less than 3% of the population of more than 100 million. The country has not reported a malaria fatality in more than 10 years (data not shown), and the number of cases of *falciparum* malaria—the most malignant species and at constant risk for importation from neighboring countries—has been reduced to zero for 3 consecutive years. Across those areas with active transmission, risk is nonuniform, with two states—Chiapas and Oaxaca—accounting for more than 75% of all reported cases in each of the past 5 years.

Risk for malaria infection in US donors deferred for travel to Mexico

Table 4 combines the results for travel destination within Mexico reported by the REDS-II donors with the proxy risk attached to the identified locations. Overall, the information provided by a large majority of donors (85%) was sufficient to identify location of travel at least to the state level. For more than half of the remaining donors (n = 69, 7.8%), information on location was either altogether lacking or insufficiently specific ("went horseback riding, visited ruins, drove through rural area") to exclude

TABLE 3. Malaria cases at national and state level and population at risk in Mexico, 2000 through 2009

	2000	2001	2002	2003	2004	2005	2006	2007	2008	2009
Population at risk (million)	50.3	54.7	54.7	54.7	2.8	2.8	2.8	2.8	2.8	=2.8
Number of states reporting ≥ 1 malaria case(s)	16	17	15	16	14	12	11	10	10	10
Number of counties reporting ≥ 1 malaria case(s)	234	202	189	177	148	133	NA	NA	115	NA
Number of *P. falciparum* cases	124	70	16	43	47	22	14	0	0	0
Individual states										
Campeche	36	57	54	21	4	1	0	0	0	0
Chiapas	3575	2522	2415	1757	1151	852	1349	1483	1136	1039
Chihuahua	695	404	420	259	184	181	122	148	185	438
Colima	0	1	0	0	0	0	0	0	0	0
Durango	171	136	104	79	52	114	121	46	38	42
Guerrero	161	88	24	7	3	0	0	0	0	0
Jalisco	50	29	11	6	5	2	2	2	10	8
Michoacán	135	52	21	2	7	0	0	0	0	0
Morelos	0	0	0	1	0	0	0	0	0	0
Nayarit	206	154	88	49	27	37	24	38	71	70
Oaxaca	654	285	260	699	1083	1432	575	369	804	896
Puebla	7	1	0	0	0	0	0	0	0	0
Quintana Roo	332	215	276	88	40	11	17	14	15	4
Sinaloa	790	616	664	377	513	208	98	108	76	72
Sonora	79	59	32	76	49	29	17	13	10	18
Tabasco	297	219	139	167	145	97	86	86	12	8
Veracruz	34	20	11	4	0	2	2	0	0	0
Yucatán	50	9	33	15	5	0	0	0	0	0
Total malaria cases	7272	4867	4552	3607	3268	2966	2413	2307	2357	2595

potential exposure to malaria, thus triggering deferral; records were no longer available for another 60 donors (6.8%). The adjusted distribution in Table 4 and the calculations based on them assume the distribution of travel by the 15% missing was random, and locations for these observations were reallocated according to the proportion missing for each blood center. Notably, more than nine of 10 donors in this analysis provided sufficient detail on their travel destination(s) within Mexico to identify both state and county visited.

Duration of exposure was estimable for 284 donors, and the distribution was bimodal. Nineteen percent of donors reported a duration of exposure of 0 days, which reflects day trips to risk areas. Another 24% reported 7-day stays in areas with malaria risk. The median and mode both were 7 days, and the mean was 7.4 days. Together, 73% of the 284 donors reported a duration of exposure of 7 or fewer days, and this duration was accordingly used to estimate the risks for malaria infection, discussed below and shown in Table 4.

A significant majority—72%—of REDS-II donors were deferred for travel to the state of Quintana Roo. Located on the Yucatán Peninsula, Quintana Roo draws heavy numbers of tourists arriving by both plane and cruise ship. As indicated in Table 3, the number of malaria cases reported in Quintana Roo has been reduced by more than 95% over the past decade, and in 2005 (Table 2) only two of the eight counties in the state had any cases. The information provided by travelers to Quintana Roo was sufficiently detailed to identify location at the county level for 98% of the donors deferred for travel to that state (Table 4). Risk for infection in donors with travel to Quintana Roo is derived from a weighted summation of location risk (county level for 98%, state level for 2%) multiplied by the number of US donors extrapolated from the REDS-II donors (Table 5). From these calculations, two figures stand out. First, 81% visited areas with zero risk for malaria in 2005 (Table 4 and Fig. 2[28]), mostly in the area of Cancún, Playa del Carmen, and other coastal areas of the northern third of the state. Second, after adjusting for duration of exposure (see above), we would expect the projected number of 47,939 donors deferred annually for travel to Quintana Roo to acquire malaria infection at a rate of 0.0080 per year, or one infection every 125 years. This estimate assumes no significant alteration in the degree or distribution of risk within the state, both of which seem reasonable inferences based on the number of cases reported statewide during 2006 through 2009 (Table 3).

After Quintana Roo, the states responsible for the second- and third-largest number of donor deferrals also represent very-low-risk travel. Both Guerrero and Nayarit account for more than 6% of travel deferrals to Mexico, but Guerrero reported no more than three cases of malaria (either zero or three, depending on the source) in 2005, and travelers to both states overwhelmingly reported travel destinations in counties with zero malaria cases throughout 2005 (see Fig. 3 for Nayarit[28]). Accordingly, the more than 8400 US donors projected to have been deferred for travel to these two states would be expected to acquire 0.0007 infections altogether, or one per 1400 years.

Continuing in like fashion across all the states responsible for donor deferrals, the results indicate that 76% of donors did not enter an area with even one malaria case

TABLE 4. Distribution of travel destination and estimated risk for acquiring malaria infection for 885 REDS-II donors deferred for malaria travel to Mexico and projections to US donors overall

State	Travel destination (%)			Location risks		Projected US deferrals and infections in donors with malaria travel to Mexico		
	Raw distribution	Adjusted distribution*	Percent assignable to county level*	Percent with travel to zero-risk area*	Estimated weighted risk, API	Number of projected US deferrals	Expected infections in number of deferred donors (and 95% CI†), 7-day exposure	Percent of total estimated risk
Quintana Roo	63.8 (n = 565)	72.0 (n = 637.5)	98	81	0.0086	47,939	0.0080 (0.0044-0.0145)	3.0
Nayarit	5.2 (n = 46)	6.5 (57.1)	89	89	0.0076	4,295	0.0006 (0.0004-0.0009)	0.2
Guerrero	4.9 (n = 43)	6.2 (55.0)	97	77	0.0010	4,140	0.0001 (0.0000-0.0006)	0.0
Oaxaca	2.3 (n = 20)	2.8 (24.6)	49	0	6.1106	1,847	0.2170 (0.1456-0.3234)	81.1
Sinaloa	1.8 (n = 17)	2.6 (22.9)	67	0	0.1253	1,751	0.0041 (0.0027-0.0064)	1.5
Jalisco	1.8 (n = 16)	2.5 (21.7)	53	53	0.0450	1,718	0.0014 (0.0002-0.0105)	0.5
Yucatán	1.8 (n = 16)	2.3 (20.3)	79	100	0.0000	1,524	0.0 (0.0-0.0)	0.0
Michoacán	1.5 (n = 13)	2.0 (17.8)	43	100	0.0000	1,342	0.0 (0.0-0.0)	0.0
11 other states and Mexico City	2.4 (n = 21)	3.2 (28.1)	78	51	0.8942	2,117	0.0364 (0.0226-0.0587)	13.6
Location unassigned	14.8 (n = 129)							
Total	885		92 (814/885)	76 (673/885)	0.2091†	66,554	0.2676 (0.2483-0.2885)	

* Adjusted distributions are reweighted according to the percent missing for each center, as shown in Table 1.
† CIs do not account for temporal or geographic variation of API, temporal variation in travel habits of US donors, or variation in donation habits of US donors.

TABLE 5. Detailed calculations for risk estimates for travel to state of Quintana Roo

County (raw/adjusted number)	Estimated resident risk, 2005	Percentage of REDS-II malaria deferrals with travel to Mexico	Number of projected US deferrals	Expected malaria infections, API (and 95% CI*), in donors (7-day exposure)
Cozumel (n = 8/8.136)	0.0000	0.92	612	0.0000 (0.0000-0.0000)
Felipe Carrillo Puerto (n = 1/1.938)	0.0306	0.22	146	0.0001 (0.0000-0.0004)
Isla Mujeres (n = 1/1.006)	0.0000	0.11	76	0.0000 (0.0000-0.0000)
Othón P. Blanco (n = 85/107.601)	0.0410	12.16	8,092	0.0064 (0.0034-0.0119)
Benito Juárez (n = 64/65.138)	0.0000	7.37	4,902	0.0000 (0.0000-0.0000)
José María Morelos (n = 0)	0.0000	0.0	0	0.0000 (0.0000-0.0000)
Lázaro Cárdenas (n = 0)	0.0000	0.0	0	0.0000 (0.0000-0.0000)
Solidaridad (n = 397/441.450)	0.0000	49.88	33,198	0.0000 (0.0000-0.0000)
Unspecified location (n = 9/12.149)	0.0861	1.37	914	0.0015 (0.0007-0.0034)
Totals		72.0	47,939	0.0080 (0.0044-0.0145)

* CIs do not account for temporal or geographic variation of API, temporal variation in travel habits of US donors, or variation in donation habits of US donors.

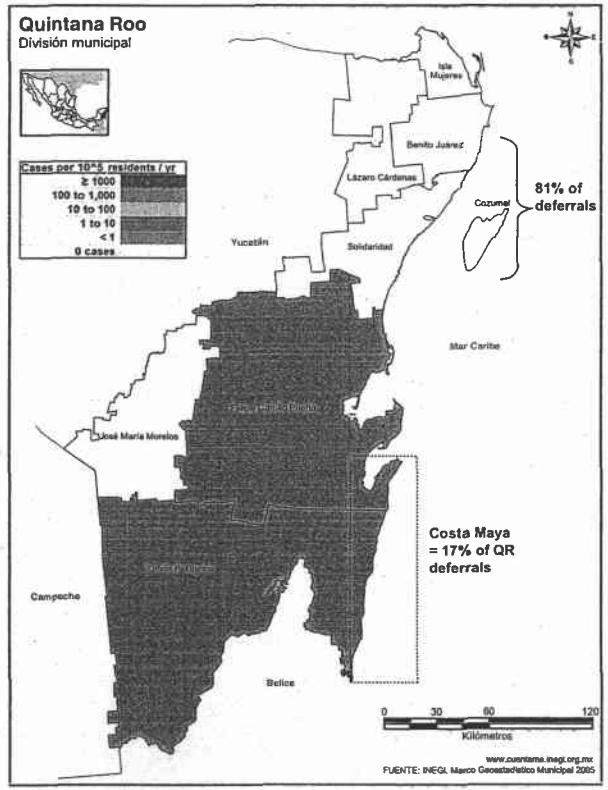

Fig. 2. Mexican State of Quintana Roo, with 2005 county-level malaria risk and general distribution of REDS-II malaria travel deferral destinations. Map adapted from Instituto Nacional de Estatística e Geografía.[28] [Correction added after online publication 12-May-2011: deferral numbers added.]

in 2005, and the weighted average location risk for all deferred donors is 0.2091 infections per 1000 year-round residents. Adjusted for duration of exposure, this estimate implies roughly a 1:250,000 malaria risk for US donors deferred for travel to Mexico. Given the 100-fold risk gradient across states shown for Mexican residents in Table 3, the donor risk categorized at the state level gives similar results. In fact, 81% of the risk attributed to donors deferred for travel to Mexico (0.2170 infections annually, or roughly one case per 4.6 years) is for travel to the state of Oaxaca, a risk associated with only 1847 donors. Omitting them from the analysis, the remaining 64,707 donors collectively incur risk for malaria infection of roughly one per 20 years. Inclusion of Oaxaca yields an estimated 0.2676 expected infections in 66,554 deferred donors per year, or roughly one infection every 3.7 years.

These frequencies can be understood as the maximum theoretical risk from fully repealing the donor deferral for travel to Mexico, which assumes that all donors present for donation immediately upon return. Given that 50% of vivax malaria infections in US travelers become symptomatic within 1 month and 70% within 3 months,[13] the actual risk under the expected distribution of intervals between return and presentation would likely be quite a bit lower. Moreover, as discussed below, imputing to US travelers a prorated risk for malaria infection equivalent to Mexican residents almost certainly overstates risk by a significant, albeit unquantifiable amount.

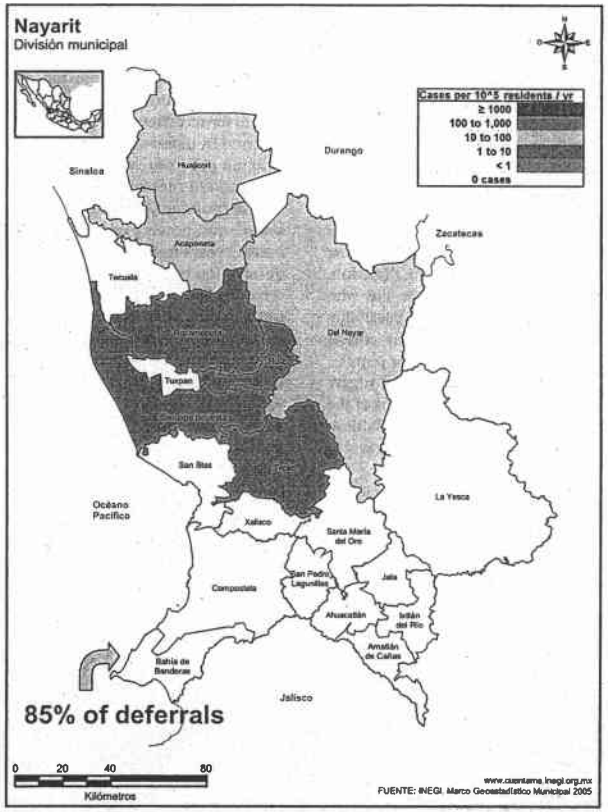

Fig. 3. Mexican State of Nayarit, with 2005 county-level malaria risk and destination location of large majority of REDS-II malaria travel deferrals. Map adapted from Instituto Nacional de Estatística e Geografía.[28] [Correction added after online publication 12-May-2011: deferral numbers added.]

It is noteworthy that donors were deferred for travel to 19 states, plus the Federal District (Mexico City), whereas only 13 states are listed by name in the Yellow Book. To be sure, these occurrences represent a small minority, but some of the states responsible for deferrals (Baja California Sur, Colima) had not reported a malaria case in several years.

DISCUSSION

While blood centers in the United States have previously expressed concern that the donor deferral for malaria risk in Mexico reflects a poor balance between risk averted and impact on blood availability,[34] no systematic effort had been undertaken to quantify either metric in a joint assessment of safety and availability. Our consortium's first analysis suggested that the risk for malaria infection associated with travel to Mexico might be low enough that the current 1-year deferral period could be scaled back with negligible impact on blood safety. Given the apparent size of the population of willing donors turned away after travel to Mexico, there are indeed significant potential gains in blood availability to be weighed in the assessment of risk and benefits that might follow any change in donor eligibility requirements. To address the potential concern that our estimates for malaria risk in US travelers to Mexico might not have been based on accurate estimates of population at risk, we sought an alternate measure for malaria risk that was independent of US traveler patterns overall. The results presented herein support the conclusions from the original paper, while providing a more nuanced perspective on donor travel patterns and attendant malaria risk.

Based on the recognition that traveler risk and donor acceptance might be only loosely associated, this analysis purposefully did not restrict its focus only to the donor population that entered an area in Mexico with active malaria transmission. While a donor who reports having entered a risk area is clearly excluded from donation, other donors who did not enter such an area but who cannot provide sufficient detail to exclude the possibility of malaria exposure are also deferred. Thus, the critical population for the current analysis is the donor population who entered an area of Mexico that would trigger their exclusion from blood donation, independent of the level of risk they might have faced. As indicated in Table 4, a large majority (76%) of donors were deferred for travel to areas reporting zero malaria cases in 2005.

The discrepancy between risk areas described in the CDC Yellow Book and actual donor deferrals could have more than one explanation. First, the deferral of donors for travel to areas with no malaria could simply reflect the natural time lag between an area within Mexico having been determined to be free of active transmission, this information being published in the next version of the Yellow Book and the newest version of the Yellow Book being integrated into blood centers' documentation for donor screening for malaria risk. Indeed, the version of the Yellow Book subsequent to the one referenced for this study removed four states from the list of those considered risk areas: Campeche, Guerrero, Jalisco, and Michoacán. A second, perhaps more important factor, might be the limited amount of detail available in the Yellow Book coupled with limited information provided by the donor him- or herself. Consider, for example, the fact that the state of Nayarit has reported nearly all its malaria from the same six counties—Huajicori, Acaponeta, Del Nayar, Rosamorada, Ruiz, and Tepic—between 2002 and 2005[30] (see Fig. 3), but 85% of REDS-II donors were deferred for visiting Nuevo Vallarta and Sayulita in the county of Bahía de Banderas in the southern part of the state, where no malaria case has been reported since at least 1999.[30] A similar dynamic prevails in the states of Guerrero and Quintana Roo. The breadth of risk implied by the phrase "risk in rural areas" followed by a long list of states implies that risk is incurred by any traveler who leaves an urban locale in any of those states, and a high lack of specificity is the result. The issue of timeliness of risk information could in theory be partly remedied by the online malaria risk map CDC has developed,[35] if subsequent iterations of this application were to provide more detailed or more recent information than the print version of the Yellow Book. In sum, while the information provided by the CDC is valuable in assessing risk, it is often insufficient to exclude the need for deferral for many presenting donors.

The risk estimates presented herein should be evaluated in terms of how realistically they reflect the actual risk incurred by deferred donors. The intent of this analysis, as with the prior one, was to develop a conservative model that ensured that risk estimates were not artificially low. We are confident that the first model is successful in this sense, most notably by its prediction of one case of TTM every 6 years from a routine traveler, whereas empirical observation has identified only one such case in the past three decades. We believe that the low risk estimates for travel to each state in the current analysis also are conservative and provide a significant margin of safety. This is probably best illustrated by the data from the state of Oaxaca, which is responsible for the largest share of cases within Mexico and the second-highest population-adjusted risk in 2005. Notably, less than 5% of the state's population lives in risk areas, and only 19 (3.3%) of the 570 counties reported cases in 2005 (Table 2). Combining census and reported malaria data,[30,31] one sees that Oaxaca's 1432 cases are scattered across 265 different towns or hamlets (of 1249 in these 19 counties), which by simple arithmetic implies an average of five malaria cases per town or hamlet, each with mean population of 700 inhabitants. Altogether, the data suggest that the population-adjusted risk of 7.39 cases per thousand residents, or 1:135 residents annually, applies to a very small share of the population and the landscape of Oaxaca. If tourists to Oaxaca faced malaria risk equivalent to those of local residents, one should expect far greater numbers of US tourists to return with malaria. Oaxaca accounts for roughly 1% to 2% of air traffic from the United States to Mexico[25,36] and is associated with 3% of the deferrals in the REDS-II cohort. If even 1% of US overnight travelers to Mexico visit Oaxaca, stay 1 week, and face the malaria risk shown in Table 4, one would expect almost 24 malaria infections from this state alone, four times the average over the past 8 years for the whole country (200,000 travelers × 1:163 risk ÷ 52 for 1-week exposure = 23.6 infections). In sum, use of local resident risk as a proxy for traveler risk undoubtedly overstates the risk in our donor population.

Whether the predicted level of risk supports a complete or partial reversion of current deferral requirements for travel to certain parts of Mexico is a question that has been reviewed recently by policy makers.[37] In a discussion focused only on Quintana Roo, Mexico, the FDA Blood Products Advisory Committee recommended 17 to 1 in favor of allowing donation from donors with travel to that state. Our analysis reinforces this conclusion and supports extending a similar policy to other areas of Mexico with exquisitely low risk for malaria infection. Both within Quintana Roo and elsewhere, most donors reported travel

to areas with zero-reported malaria cases in 2005, and those that traveled to areas with theoretical risk generally incurred risks of very low magnitude. Specifically, three in four deferred donors were associated with areas that were malaria-free in 2005, and 85% of the remainder faced estimated 1-week risks of less than 1:640,000. Only the 3.5% of donors deferred for travel to Oaxaca, Chihuahua, and Sonora faced potential risks that could be considered nontrivial. This finding replicates that from our consortium's first analysis, albeit here on a subnational rather than global scale, the largest share of malaria travel deferrals is associated with travel to the area of lowest estimated risk. Just as Mexico reflects about a 1000-fold lower risk than Africa, donors who visited Quintana Roo appear to have faced an average 1 in 115,661 (annualized) risk, compared to travelers to Oaxaca, with its estimated risk of 1 in 164, a 700-fold difference. Adjusting each for a 1-week exposure, that leaves us with absolute risks on the order of 1 per 6 million for Quintana Roo versus 1 per 8510 for Oaxaca. Returning again to the earlier article, the absolute traveler risk for Quintana Roo is less than 8000 times that of the estimated traveler risk to Africa. We believe that at a minimum Quintana Roo should no longer be considered a deferrable travel destination, and strong consideration should be given to extending such a measure to other locations in Mexico with very low malaria risk.

This study is subject to a number of potential limitations. First, donors from the REDS-II blood centers might have travel patterns that are not representative of US donors overall. In fact, given the lack of REDS-II data from the southwestern United States, one might assume that at least that region's donors could have a different amount of travel to Mexico or undertake travel of a different nature compared to REDS-II donors. While such data are sparse, a parallel study to this one was presented to the FDA's Blood Products Advisory Committee. Using 2008 deferral data from blood centers in the border states of Texas, Arizona, and New Mexico, the study found a similar percentage of donors deferred for malaria travel as was found in REDS-II donors (0.9% vs. 1%), but found that a higher percentage were deferred for travel to Mexico (60% vs. 42%). The distribution across states differed from that in REDS-II donors, with more deferrals for travel to border states Chihuahua and Sonora within Mexico (15% vs. <1%); however, Quintana Roo still represented the state responsible for the largest share (37%) of deferrals. More broadly, the study suggested that a majority of border state donors are also deferred for visits to areas with very low risk (<1:250,000 assuming 1-week exposure).[38]

Another potential limitation is that location data were not available from 15% of the donors in this study who were deferred for travel to Mexico. To cause downward bias in our risk estimates, however, the missing data would have to be from donors with higher malaria risk while traveling to Mexico than other donors from the same blood center, an unlikely scenario. If anything, the missing data are likely to come from relatively low-risk travelers. Since by 2008, 100% of malaria in Mexico has been characterized as occurring in indigenous populations in scattered rural areas,[24] one should expect these areas to be visited by the more adventurous and/or sophisticated traveler, who almost certainly would be able to name at least the state they visited. In any case, because the missing data are adjusted for by reweighting the available data for each center, any difference in risk for donors across REDS-II centers is adequately accounted for.

A third concern might be that the reports of lower levels of malaria within Mexico could reflect diminished surveillance capacity rather than reduced malaria transmission. Available evidence, however, suggests this is not the case. Mexico accounts for less than 0.5% of malaria cases diagnosed across all endemic countries in the Americas, yet carries out more than 18% of all blood smears examined for diagnostic purposes.[39] A slide positivity rate that is a fraction of other countries' together with a higher proportion (36% vs. 21%)[40] of cases found through active case detection confirms that Mexico's malaria surveillance is both robust and committed to identifying all potential cases of infection.

One final possible limitation is that our estimates for risk and benefits associated with modifying the deferral guidelines to Mexico are based on risk estimates from 2005 only, and detailed county-level data are not available from 2006 onward. This could have implications for the reliability of the estimates presented herein as well as for inferences applied to subsequent time periods. While two-thirds of the donors in our analysis actually traveled in 2006, we have no a priori reason to assume that reliance on 2005 malaria surveillance data prejudices our risk estimates. First, between 2005 and 2006, the overall number of cases declined from 2966 to 2413, the API declined from 1.06 to 0.90,[26] and three of the four states with the highest API in 2005 all reported fewer cases in 2006: Chihuahua from 181 in 2005 to 122 in 2006, Oaxaca from 1432 to 575, and Sonora from 29 to 17; Durango, ranked highest in risk in 2005, was only slightly higher in 2006, increasing from 114 to 121 cases. Going forward, one might be concerned that the lack of county-level data beyond 2005 could mean that any relaxation of deferral guidelines might not have recent surveillance data of a high level of granularity to support it. While the ideal scenario would involve the availability of real-time, town- or hamlet-level data, the data presented here are part of a long, downward trend in risk for malaria in Mexico, and most of the areas shown to be malaria-free (or very low risk) in 2005 were also malaria-free or low-risk for several prior years. Further, malaria case reports at the state level are currently available on a weekly basis, with only a 2- to 3-week time lag,[31] so that any significant change in malaria transmission would likely become rapidly detectable. Unless the nature of donor travel patterns to Mexico were to be reoriented toward risky travel in remote areas and away from beach resorts, the estimates of risk described in this analysis are likely to remain relatively stable.

Based on the preponderance of blood donor deferrals triggered by visits to areas of zero or very limited risk for malaria, we recommend that the deferral policies for donor travel to Mexico be revised in a way that balances risk averted with the significant donor loss. While multiple permutations of new regulations might reasonably be considered, we believe that the data from this analysis and available online are sufficient to inform these efforts.

APPENDIX

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

Blood centers:

American Red Cross Blood Services, New England Region: R. Cable, J. Rios, and R. Benjamin

American Red Cross Blood Services, Southern Region/Department of Pathology and Laboratory Medicine, Emory University School of Medicine: J.D. Roback

Hoxworth Blood Center, University of Cincinnati Academic Health Center: R.A. Sacher, S.L. Wilkinson, and P.M. Carey

Blood Centers of the Pacific, University of California San Francisco, Blood Systems Research Institute: E.L. Murphy, B. Custer, and N. Hirschler

The Institute for Transfusion Medicine: D. Triulzi, R. Kakaiya, and J. Kiss

BloodCenter of Wisconsin: J. Gottschall and A. Mast

Coordinating center:

Westat, Inc.: J. Schulman and M. King

National Heart, Lung, and Blood Institute, NIH

G.J. Nemo

Central laboratory:

Blood Systems Research Institute: M.P. Busch and P. Norris

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CONFLICT OF INTEREST

The authors have no conflicts of interest or other financial involvement to declare.

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39. Pan American Health Organization. Malaria surveillance data. Table 4: Total blood slides examined and number of positive slides by level of malaria transmission, 2007. [cited 2011 Feb 25]. Available from: URL: http://www.paho.org/English/ad/dpc/cd/mal-americas-2007.pdf
40. Pan American Health Organization. Malaria surveillance data. Table 6: Comparison between passive and active case detection, 2006. [cited 2011 Feb 25]. Available from: URL: http://www.paho.org/English/ad/dpc/cd/mal-americas-2007.pdf

別紙様式第2−1

No. 15

医薬品　研究報告　調査報告書

識別番号・報告回数			報告日	第一報入手日	新医薬品等の区分	総合機構処理欄
				2011. 11. 14	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	European Centre for Disease Prevention and Control; News 28 October 2011	公表国	
販売名（企業名）	新鮮凍結血漿-LR「日赤」(日本赤十字社) 新鮮凍結血漿-LR「日赤」成分採血（日本赤十字社）				ヨーロッパ	

研究報告の概要	○ギリシャでのマラリア　2011年10月 2011年5月21日～10月26日の間に61症例の三日熱マラリア感染がギリシャで報告された。これらの症例のうち33例は流行国への旅行歴がないギリシャ市民で、そのうち27症例は南ギリシャのLakonia県にあるEvrotasから報告された。加えて、季節労働者の28症例もEvrotasから報告され、これらの移民の大部分（21人）はマラリア流行国の出身者であった。 Evrotasでの最初の報告症例は5月23～29日の週に発症し、最後の2症例は10月17～23日の週に発症した。報告された症例のピークは9月5～18日の週の間であった。全ての症例が三日熱マラリアと確認され、基礎疾患を持つ70歳代男性の1死亡例を除き、全員軽症であった。 10月上旬から当該地域の気温は20℃以下になり、媒介蚊数は急速に減少している。疫学的・昆虫学的な情報や、冬の季節の到来により、現在、当該地域における感染の勢力は非常に弱いと考えられ、まもなく終息すると予測される。この理由により、当該地域への訪問者に対するマラリアの予防服薬は必要ないと考えられる。しかし蚊の刺咬予防の為の標準的な方法の使用促進は継続される。
報告企業の意見	ギリシャで2011年5月～10月の間に61症例の三日熱マラリア感染が報告されたが、まもなく終息する見通しであるとの報告である。
今後の対応	日本赤十字社では、輸血感染症対策として問診時に海外滞在歴の有無を確認し、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、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。
使用上の注意記載状況・その他参考事項等	新鮮凍結血漿-LR「日赤」 新鮮凍結血漿-LR「日赤」成分採血 血液を介するウイルス、細菌、原虫等の感染 vCJD等の伝播のリスク

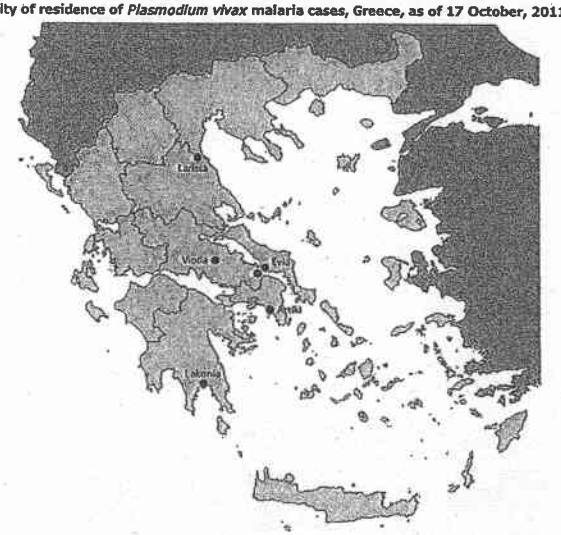
Epidemiological update: Malaria in Greece, October 2011

28 Oct 2011

According to the available epidemiological and entomological information, and the arriving winter season, the intensity of malaria transmission in Evrotas, Lakonia in Greece is believed to be very low and is expected to cease shortly. For this reason, chemoprophylaxis for malaria is not recommended for visitors to the area. The use of standard mosquito biting prevention measures continues to be encouraged.

Between 21 May and 26 October, 2011, 61 cases of *Plasmodium vivax* infection have been reported in Greece. Thirty-three of these cases were Greek citizens without travel history to an endemic country. Twenty seven cases are reported from the area of Evrotas, a 20 km2 river delta area, located in the district of Lakonia in Pelloponese, southern Greece (see map). The remaining six cases are from the municipalities of Attiki (n=2), Evoia (n=2), Viotia (n=1) and Larissa (n=1). In addition, 28 cases of *P. vivax* infection in migrant workers have been reported from the area of Evrotas. Of the 28 malaria cases identified in immigrants in Evrotas, Lakonia, the majority (n=21) originated from malaria endemic countries even though a clear importation status could not be determined; 19 originated from Pakistan and two from Afghanistan. For the remaining seven immigrant cases, five were from Romania (two were diagnosed in Romania), one from Morocco, and one from Poland. It should be noted that all reported cases in immigrants (including the two cases diagnosed in Romania) are in persons that work in the agricultural areas in this part of Greece.

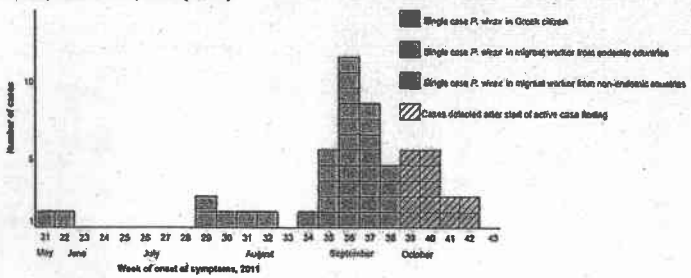
Map: Municipality of residence of *Plasmodium vivax* malaria cases, Greece, as of 17 October, 2011 (n=61)



The first reported cases in Evrotas had symptom onsets in epidemiological week 21 (23-29 May) and the last two cases had reported symptom onset during week 42 (17 – 23 October). A peak in reported symptom onset occurred during weeks 36 and 37 (5 – 18 September). Since then a steady decline in reported cases has been observed despite the ongoing active case finding in the area. All cases reported from other areas in Greece report symptom onset before September 2011. Cases reported in the last two weeks would have likely been infected during September or early October. All cases are confirmed *P. vivax* infections and all were mild, apart from one fatal case in a male aged over 70 years who had pulmonary co-infection and underlying medical conditions.

Data from entomological surveillance indicates that there have been very low larval densities detected in mosquito breeding sites and an absence of adult *Anopheles* mosquitoes captured in carbon dioxide light traps in Evrotas, Lakonia in recent weeks. Since early October, temperatures in the area have dropped below 20°C and the vector population is rapidly waning as expected for autumn and winter months.

Figure: Distribution of reported cases of P. vivax infection by date of onset and citizenship status, Lakonia, Greece, week 21-43, 2011 (n=53).



reported case was a migrant from Afghanistan who arrived in Greece during the previous 20 days.

Control measures implemented include enhancing surveillance in the affected areas to rapidly identify cases; active case finding in Evrotas, Lakonia (house to house visits since 1 October 2011) followed by microscopy diagnosis for malaria and supervised treatment according to the national protocol (chloroquine and primaquine for uncomplicated *P. vivax* infection); informing health care professionals on early malaria diagnosis and treatment; raising public awareness, and strengthening blood safety. A decree was issued by the Greek MOH to facilitate prompt diagnosis and treatment. Intensified mosquito control measures have also been carried out by local authorities. All *P. vivax* infections were sensitive to chloroquine.

Malaria is an infectious disease caused by the *Plasmodium* parasite, and transmitted by *Anopheles* mosquitoes. The incubation period varies between 7 and 15 days, but long incubation periods of several months (and years) have been observed for P. *vivax* malaria. Malaria is characterised by fever and influenza-like symptoms, including chills, headache, myalgia, and malaise; these symptoms can occur at intervals. More information can be found on the ECDC factsheet.

Following the two visits of ECDC and WHO experts to the affected areas, ECDC has not changed its risk assessment of this event since October 11, 2011, and considers the current risk for malaria infection in Greece to be to persons residing and/or working in the affected areas of Greece, particularly that of Evrotas, Lakonia. This is a geographically delimited area, having a small population and is not a touristic destination. According to the available epidemiological and entomological information, and the arriving winter season, the intensity of malaria transmission in Evrotas, Lakonia is believed to be very low and is expected to cease shortly. For this reason, chemoprophylaxis for malaria is not recommended for visitors to the area. The use of standard mosquito biting prevention measures continues to be encouraged.

Read more:
ECDC Rapid Risk Assessment malaria Greece 2011.
Eurosurveillance article describing the outbreak
More information is available on KEELPNO website
ECDC factsheet

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
				2012. 3. 15	該当なし	
一般的名称	解凍人赤血球濃厚液		研究報告の公表状況	Eurosurveillance Edition 2012: Volume 17/ Issue 10; Available from: http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=20114	公表国 ドイツ	
販売名(企業名)	解凍赤血球濃厚液「日赤」(日本赤十字社) 照射解凍赤血球濃厚液「日赤」(日本赤十字社) 解凍赤血球-LR「日赤」(日本赤十字社) 照射解凍赤血球-LR「日赤」(日本赤十字社)					

研究報告の概要

○ケニヤのマサイマラ地区から帰国したドイツ人旅行者におけるローデシアトリパノソーマ感染

2012年1月、ケニヤのマサイマラ地区から帰国したドイツ人旅行者においてヒトアフリカトリパノソーマ症(HAT)が確認された。この患者は62歳男性で、1月18、19日にマサイマラ国立保護区を訪れ、キャンプ及びサファリ観光をした。大半の時間を半袖半ズボンで過ごし、昆虫忌避剤を使用していた。帰国後の1月28日に発熱により入院し、2月1日に厚層血液スメア標本のギムザ染色で*Trypanosoma brucei rhodesiense*が確認された。スラミンによる治療が行われ、回復した。この症例の1カ月後に、マサイマラ地区からの輸入HAT症例がもう一件報告された。過去10年にわたり、マサイマラ地区から報告されるHAT症例がなかったため、これは注目すべき報告である。この報告は、関連する地域からの旅行者を扱う臨床医に、HATへの認識を促すものとなるであろう。

使用上の注意記載状況・その他参考事項等

解凍赤血球濃厚液「日赤」
照射解凍赤血球濃厚液「日赤」
解凍赤血球-LR「日赤」
照射解凍赤血球-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

12

報告企業の意見	今後の対応
ケニヤのマサイマラ地区から帰国したドイツ人旅行者にローデシアトリパノソーマ感染が確認されたとのことである。 なお、この情報に関して米国CDCからこの地区への旅行者に向けたトラベルアラートが発出されているほか、日本の厚生労働省検疫所のホームページでも情報提供がされ、注意が呼びかけられている。	日本赤十字社では、輸血感染症対策として受付時に海外滞在歴の有無を確認し、帰国(入国)後4週間は献血不適としている。また、アメリカトリパノソーマ症の既往がある場合には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。

MedDRA/J Ver.15.0J

RAPID COMMUNICATIONS

Trypanosoma brucei rhodesiense infection in a German traveller returning from the Masai Mara area, Kenya, January 2012

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Article published on 8 March 2012

In January 2012, a case of Human African Trypanosomiasis (HAT) has been identified in Germany in a traveller returning from the Masai Mara area in Kenya. The 62-year-old man had travelled to the Masai Mara game park from 18 to 19 January 2012 and developed fever on 28 January. The infection with *Trypanosoma brucei rhodesiense* was confirmed by laboratory testing three days hereafter.

Case report

On 28 January 2012, a 62-year-old man was hospitalised after a sudden onset of fever with temperature up to 39°C in a local hospital near Frankfurt, Germany. The fever started after his return from a holiday trip to Kenya from 8 to 28 January. Upon arrival in Germany and admittance to a local hospital, the patient was suspected to have malaria and treatment with Atovaquon / Proguanil was administered for two consecutive days. The diagnosis was made on the basis of a thin smear, which was later re-evaluated after the patient's transfer to the Infectious Diseases Department of Frankfurt University Hospital and no Plasmodium parasites were detected.

He had travelled by airplane directly from Frankfurt to Mombasa and back and spent all the time at a beach resort south of Mombasa except for a trip to the Masai Mara area from 18 to 19 January. For this trip, he flew from Mombasa to the Ol Kiombo airstrip, stayed at a camp in the area, and then went on safari excursions within a radius of approximately 50 km from the camp. He wore shorts and short sleeved shirts most of the time and used insect repellents.

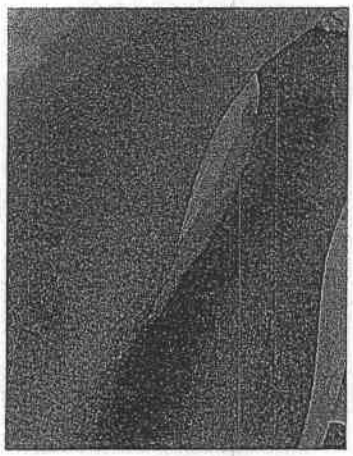
Despite anti-malarial treatment, the patient was still febrile on 31 January and was transferred to the Infectious Diseases Department of Frankfurt University Hospital. By then, the clinical symptoms had become more severe, with strong frontal headaches, vertigo, nausea and arthralgia. Fever was still high at 39.1°C. He had two distinct, painless skin lesions over both tibiae (Figure 1), but no localised or disseminated lymph node enlargement.

Malaria parasites were not confirmed in Quantitative Buffy Coat, Giemsa-stained thin or thick blood smears and the malaria antigen test (BinaxNow) was negative. However, *Trypanosoma brucei rhodesiense* was detected in thick blood smears stained with Giemsa (Figure 2) on 1 February.

Treatment was started three hours after diagnosis of trypanosomiasis with 1 g of suramin as a continuous infusion over one hour. As the substance was not readily available, it was brought to Frankfurt University Hospital from the "Missionsärztliche Klinik" Würzburg, Germany, where a regular stock of suramin is kept. In parallel, the patient was given prednisolone to prevent

FIGURE 1

Chancres due to infection with *Trypanosoma brucei rhodesiense* in a German traveller returning from the Masai Mara area, Kenya, January 2012



allergic reactions. The treatment was followed on day 1, 3, 7, 14 and 21 without complications.

A lumbar puncture performed on day 2 of therapy revealed a normal cerebrospinal fluid (CSF) pattern and a PCR with *Trypanosoma spp.* specific primers was negative from CSF as opposed to the peripheral blood, where it was found to be positive. The patient had leuko- and thrombopenia, an elevated complement regulatory protein (CRP) and aspartate and alanine transaminase levels two times the upper limit of normal. Electrocardiogram and echocardiography did not show any pathological findings.

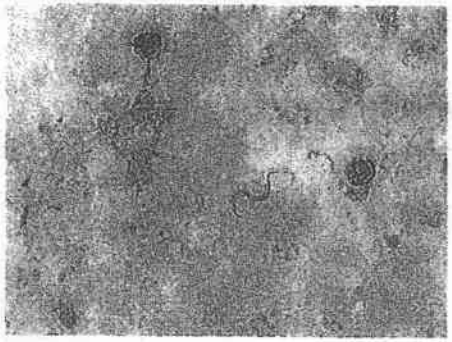
The fever subsided on day 2 of treatment and no parasites were detected from day 3 of the treatment onwards. *T. b. rhodesiense* antibodies were detected by immunofluorescence testing performed at the reference laboratory (Bernhard Nocht Institute, Hamburg, Germany) on day 8 of treatment, 12 days after the first symptoms whilst having been negative on day 1 of treatment. The patient concluded his treatment as planned on day 21 without any residual problems and left the hospital.

Discussion

Following the detection of a case of Human African Trypanosomiasis (HAT) we screened the literature for recent alerts of HAT in Kenya and only ProMED had previously published a report on the occurrence of HAT in Kenya. This however, was documented almost 11 years before the current case [1,2]. About a month after the occurrence of the case described here, there was a further case of HAT from the Masai Mara area described in this issue of *Eurosurveillance* [3].

Figure 2

Giemsa-stained *Trypanosoma brucei rhodesiense* in a thick blood smear from a German traveller returning from the Masai Mara area, Kenya, January 2012



64x magnification

A literature research on PubMed revealed two publications that reviewed the epidemiology of HAT in non-endemic countries. A review of HAT cases imported into Europe between 2005 and 2009 included 11 cases, five of which were infected with *T. b. rhodesiense*. There were no cases described from Kenya, but two infected patients had travelled to the Serengeti, which directly borders Masai Mara [4]. In another report, the bibliographic data were supplemented by the World Health Organization (WHO) data on requests of antitrypanosomal drugs from hospitals in non-endemic countries treating travellers. These data showed that 94 cases of HAT were identified between 2000 and 2010, 72% of which were caused by *T. b. rhodesiense*. Although 59% of the cases were identified in Tanzania, with the vast majority of cases being tracked back to the Serengeti, no cases have been reported from Kenya [5].

Trypanosomiasis is a disease that occurs in local clusters, and one such cluster was identified in 2002 through the TropNetEurop Sentinel Surveillance network when two index and seven consecutive cases were identified in non-disease endemic countries in Europe and South Africa [6]. These cases originated in the Serengeti and Tarangire National Parks in close proximity to Masai Mara, but with no documented case originating from the latter.

The above mentioned reports documented imported cases that were diagnosed in non-endemic countries. There are data on the cases diagnosed within the country however. The Kenyan reference hospital for sleeping sickness in Alupe, which is on the Ugandan border north of Lake Victoria, reported 31 patients with HAT caused by *T. b. rhodesiense* between 2000 and 2009. Twenty-two of the patients were diagnosed at the late stage of the diseases and coinfections and comorbidities were frequent [7]. Additionally, WHO extensively mapped the epidemiology of HAT in Africa between 2000 and 2009. For Kenya, sporadic cases were described in the very western provinces Bungoma, Teso and Busia, again on the Ugandan border, as well as in the Nyanza province. Epidemiological analysis of HAT in Kenya between 1950 and 2007 showed that infections occurred exclusively in these Western provinces, and the prevalence is altogether estimated to be low with only sporadic infections the 1990s onwards [8, 9].

Conclusion

We identified a case of HAT due to *T. b. rhodesiense* infection in a traveller who had returned from the Masai Mara area, Kenya. After this case, another report of an imported HAT infection from this area was diagnosed one month later and communicated worldwide [10]. This is noteworthy, as there were no cases described from Masai Mara in the last decade. Previously, there was documented disease activity in Kenya which was limited to the western provinces, as well as Serengeti which is essentially in direct vicinity to Masai Mara. This report should alert clinicians to be aware of HAT when dealing with travellers from the area concerned.

We have been in contact with WHO in Geneva, Switzerland, who confirmed that the local authorities in Kenya have been informed and a WHO team of experts has been sent to the area to elucidate the situation.

Acknowledgments

We would specifically like to thank Prof. Dr. med. August Stich, affiliated with the "Missionsärztliche Klinik Würzburg, Germany" for his generous and swift support in providing us with suramin. The authors would also like to thank all the medical staff and the diagnostic team involved in the treatment and diagnosis. We would like to extend our gratitude to the patient for agreeing to this publication.

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No. 18

医薬品 研究報告 調査報告書

識別番号・報告回数		報告日	第一報入手日	新医薬品等の区分	総合機構処理欄
			2011. 11. 17	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	Cancelas JA, Dumont LJ, Rugg N, Szczepiorkowski ZM, Herschel L, Siegel A, Pratt PG, Worsham DN, Erickson A, Propst M, North A, Sherman CD, Mufti NA, Reed WF, Corash L.. Transfusion. 2011 Nov;51(11):2367-76. doi: 10.1111/j.1537-2995.2011.03163.x.	公表国 米国	
販売名(企業名)	新鮮凍結血漿-LR「日赤」(日本赤十字社) 新鮮凍結血漿-LR「日赤」成分採血(日本赤十字社)				

研究報告の概要

○第2世代S-303病原体不活化処理後の保存赤血球の生存率

背景: 輸血感染症は赤血球(RBC)輸血における懸念事項である。RBC製剤のための病原体不活化技術が輸血の安全性をより高めるために開発中である。S-303はfrangible anchor-linker-effector(FRALE)複合体であり、アルキル化により強力に病原体を減少させる。この研究において、第2世代S-303プロセス処理後35日間保存したRBCの生存能力を調査した。

研究デザイン及び方法: 27人の健康人より得られたRBCをS-303による不活化またはコントロールにランダム割り付けして処理し、35日間保存した。放射標識したRBCを被験者に戻し輸血し、24時間回収率、RBC生存期間を測定した。またin vitroでの代謝・生存能力の変化を分析した。

結果: S-303処理RBCの輸血24時間後回収率及びin vitroの溶血率はコントロールと同等で、FDAの基準を満たしていた。コントロールと比べて生存率は13.7日、T50生存率の中央値は6.8日短かったが、RBC生存曲線より下の領域の面積の差は1.38%にとどまった。不活化処理RBC輸血後、臨床的に意味のある検査値の異常は認められなかった。処理RBCの自己クロスマッチはすべて陰性であった。

結論: S-303病原体不活化プロセスで処理されたRBCは35日間の保存後、生理学的にも代謝的にも輸血に適しており、24時間回収率もFDAガイダンスを満たし、抗体産生も誘発しなかった。

使用上の注意記載状況・その他参考事項等

新鮮凍結血漿-LR「日赤」
新鮮凍結血漿-LR「日赤」成分採血

血液を介するウイルス、細菌、原虫等の感染
vCJD等の伝播のリスク

13

報告企業の意見	今後の対応
第2世代S-303病原体不活化処理した赤血球について保存35日後に調査したところ、生体内回収率は輸血に適する状態であったとの報告である。	今後も細菌やウイルスを不活化する方策について情報の収集に努める。

MedDRA/J Ver.14.1J

JRC2011T-040

BLOOD COMPONENTS

Stored red blood cell viability is maintained after treatment with a second-generation S-303 pathogen inactivation process

Jose A. Cancelas, Larry J. Dumont,* Neeta Rugg, Zbigniew M. Szczepiorkowski, Louis Herschel, Alan Siegel, P. Gayle Pratt, D. Nicole Worsham, Anne Erickson, Meisa Propst, Anne North, Claire D. Sherman, Nina A. Mufti, William F. Reed, and Laurence Corash*

BACKGROUND: Transfusion-transmitted infections and immunologic effects of viable residual lymphocytes remain a concern in red blood cell (RBC) transfusion. Pathogen reduction technologies for RBC components are under development to further improve transfusion safety. S-303 is a frangible anchor-linker-effector with labile alkylating activity and a robust pathogen reduction profile. This study characterized the viability of RBCs prepared with a second-generation S-303 process and stored for 35 days.

STUDY DESIGN AND METHODS: This was a two-center, single-blind randomized, controlled, crossover study in 27 healthy subjects. S-303 (test) or control RBCs were prepared in random sequence and stored for 35 days, at which time an aliquot of radiolabeled RBCs was transfused. The 24-hour recovery, RBC life span, and in vitro metabolic and viability variables were analyzed.

RESULTS: The mean 24-hour RBC recovery and hemolysis of test RBCs were similar to control RBCs and were consistent with the Food and Drug Administration (FDA) guidance for RBC viability. The mean differences in life span and median life span (T_{50}) of circulating test RBCs were 13.7 and 6.8 days, while the mean difference in the area under the curve of surviving RBCs was 1.38%, in favor of control RBCs. There were no clinically relevant abnormal laboratory values after the infusion of test RBCs. All crossmatch assays of autologous S-303 RBCs were nonreactive.

CONCLUSIONS: RBCs prepared using the S-303 pathogen inactivation process were physiologically and metabolically suitable for transfusion after 35 days of storage, met the FDA guidance criteria for 24-hour recovery, and did not induce antibody formation.

Transfusion-transmitted diseases (TTDs) persist despite implementation of screening tests and donor deferral policies. Blood donations within the window period of detection, containing a low pathogen copy number below the limit of detection of current screening tests, and emergence of new pathogens for which licensed tests are not available are the main causes of TTD persistence.[1-4] Inhibition of nucleic acid replication in cellular blood components inactivates pathogens and viable residual white blood cells (WBCs) associated with TTD and adverse immune-mediated reactions.[5,6] The current prevention strategy for immunologic effects, including transfusion-associated graft-versus-host disease prevention, is through leukoreduction and ionizing irradiation treatment of blood components.[7] However, this treatment is associated with a reduction in red blood cell (RBC) shelf life and the release of high levels of potassium into the extracellular medium[8] and requires two blood component inventories in hospitals that do not practice universal irradiation of all blood components.[9]

ABBREVIATIONS: AE(s) = adverse event(s); AUC(s) = area(s) under the curve; GSH = glutathione; MCHC = mean corpuscular hemoglobin concentration; TTD(s) = transfusion-transmitted disease(s).

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TRANSFUSION 2011;51:2367-2376.

Immunologic complications of transfusion, including transfusion-related alloimmunization and immunomodulation, have been associated with WBC antigens and WBC-derived compounds. While implementation of universal leukoreduction has resulted in a significant decrease in infectious complications,[10] residual WBCs are believed to be a major source of alloimmunization and immunosuppression after transfusion.[11-13] Some biologic data have suggested that use of pathogen inactivation methods may reduce alloimmunization associated to WBC antigen presentation.[14-17]

Treatment of RBCs with S-303 has been shown to effectively (≥4 log) reduce a wide range of blood-borne pathogens and inactivate residual WBCs.[18-22] S-303 contains an intercalator group that inserts into the helical region of the nucleic acid, an effector group that allows covalent modification of nucleic acid, and a central frangible bond that allows degradation of the compound. Activation and cross-linking occurs on transfer from a low pH environment to the neutral pH of blood. The process is completely light independent. The primary byproduct of S-303 degradation is S-300, a nonreactive species that is excreted. Glutathione (GSH), a naturally occurring substance present in most cells of the body at millimolar concentrations, is used in conjunction with the S-303 treatment to quench nonspecific reactions of S-303 such as surface modification of the RBC.

The first-generation S-303 treatment process for RBCs (0.2 mmol/L S-303 and 2 mmol/L GSH) was evaluated in a series of six clinical studies. Four of these studies were radiolabel recovery studies in healthy subjects,[23,24] and two were Phase III studies in patients requiring therapeutic RBC transfusion support.[25,26] The Phase III clinical study of chronic RBC transfusion was terminated before completion when antibodies to S-303–treated RBCs were detected in two patients requiring chronic RBC transfusion support.[27] To eliminate the immunoreactivity of the treated RBCs observed in the Phase III study, the S-303 treatment process was modified to reduce the amount of acridine bound to RBCs by increasing the concentration of the quencher, GSH, to 20 mmol/L and increasing the pH of the GSH by using a disodium salt form.

An initial in vivo kinetic study with RBCs treated under these modified conditions showed a reduced packed cell volume due to moderate dehydration and reduced 24-hour RBC recovery of S-303–treated RBC stored for 35 days compared to controls.[24] The S-303 treatment process was then optimized by replacing the hypertonic GSH containing supernatant after treatment with a standard, isotonic solution before storage. This report describes the in vitro characterization and viability of RBC prepared with this second-generation S-303 treatment process that incorporates the process modifications of treatment with higher pH and GSH concentration followed by a volume exchange step before storage.

MATERIALS AND METHODS

Study design and enrollment

This was a randomized, single-blind, controlled, two-period crossover study, designed to evaluate 24-hour post-transfusion recovery and life span in healthy adult subjects of autologous RBCs prepared using the second-generation S-303 treatment process and stored for 35 days. A total of 27 subjects were enrolled and consented using an informed consent document approved by the local institutional review board, at two study sites, the Hoxworth Blood Center, University of Cincinnati, and Dartmouth-Hitchcock Medical Center. The order in which each subject was transfused with S-303–treated (test) and control RBCs was randomly assigned at the time of enrollment. Each treatment period consisted of autologous blood donation, preparation of RBCs according to treatment assignment, infusion of radiolabeled autologous RBCs, and collection of blood samples for assessment of RBC recovery and life span (Fig. 1).

The primary endpoint was comparison of the 24-hour posttransfusion RBC recovery. Additional endpoints were mean life span, median life span (T_{50}), and area under the RBC clearance curve (AUC); the incidence of anti–S-303 development after transfusion of test RBCs (with or without S-303 RBC specificity) measured using a gel card crossmatch assay against S-303 RBCs; incidence of adverse events (AEs); and the in vitro characteristics of test and control RBCs.

Donor eligibility criteria included age 18 years or older, meeting AABB physical exam guidelines for allogeneic blood donation, no prior S-303 exposure, negative direct antiglobulin test, a predonation blood hemoglobin (Hb) level of 13.0 g/dL for females and 14.5 g/dL for males, and healthy by history and physical examination.

Sample size was determined to reach 80% power and detect a treatment difference of 4.4% (absolute difference) and 5.2 days in RBC recovery and T_{50} at the two-sided 0.05 significance level, assuming a standard deviation (SD) of 7.5% and 8.8 days for the paired difference in RBC recovery and T_{50}, respectively, based on a previous crossover study of S-303 RBC recovery and life span.[24]

Preparation of S-303 RBC and control components

On Day 0 of Treatment Periods 1 and 2, 1 unit of autologous whole blood (500 ± 50 mL) was drawn from the subject into a primary collection container containing citrate-phosphate-dextrose anticoagulant (Terumo Imuflex WB-RP blood bag system with in-line leukoreduction filter, Terumo Corp., Tokyo, Japan). Collected blood was leukoreduced and processed by centrifugation into RBCs by removing plasma and adding AS-5 (Optisol, Terumo Medical Corporation, Somerset, NJ) to achieve a final hematocrit (Hct) ranging from 50% to 65%. Both test and control RBCs were shipped to Cerus Corp. where the test RBCs were treated with the S-303 treatment process (Fig. 2) within 5 days after donation, and the control RBCs were untreated. All units were stored at 1 to 6°C in monitored refrigerators until return shipment to the study sites. All shipments were on wet ice, maintaining temperatures of 1 to 10°C.

Test RBCs were prepared by diluting RBCs in a solution containing 55 mmol/L dextrose, 1.3 mmol/L adenine, 55 mmol/L mannitol, and 20 mmol/L sodium citrate, to reach a Hct of approximately 40% in a closed processing set (Avail Medical, Irving, TX). GSH sodium salt (TAD 2500, BioMedica Foscama, Ferentino, Italy) and S-303 (AMRI, Albany, NY, and KP Pharmaceutical Technologies, Bloomingdale, IN) dissolved in 0.9% saline for injection was used. Approximately 15 mL of each was added to achieve final concentrations of 20 and 0.2 mmol/L, respectively. The RBCs were transferred to an incubation and storage container and stored at 20 to 25°C for approximately 18 hours. After being incubated, the RBC units were centrifuged for 6 minutes at 4100 × *g*, and approximately 250 mL of the supernatant was removed and replaced with 100 mL of AS-5, followed by storage at 1 to 6°C. Each RBC unit was returned to the respective study site within 14 days after S-303 treatment. Unlike the first generation of S-303 process,[23] the use of an exchange process avoided the need for a compound adsorption device.

Because the study RBCs had been out of their control when shipped off-site, each study site confirmed and documented that the study RBC units returned to them

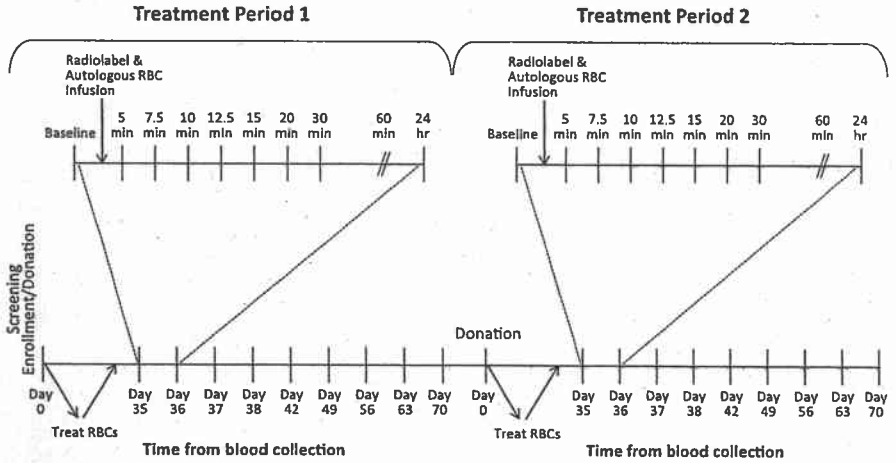

Fig. 1. Schema of the study design. Subjects were enrolled in two treatment periods (70 days each) where autologous control or test RBCs, randomly, were radiolabeled and infused.

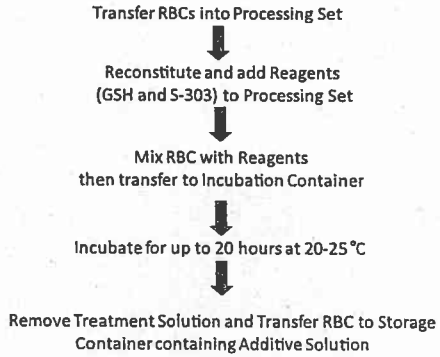

Fig. 2. Diagram of the treatment configuration used to prepare S-303 RBCs. The RBC unit and diluents solution are transferred to the mixing container of the processing set. The GSH and S-303 are reconstituted and then sterilely transferred to the mixing container of the processing set resulting in a final concentration of 0.2 mmol/L S-303 and 20 mmol/L GSH. After being mixed with GSH and S-303 with the RBC and diluent solution, the treated RBC unit is transferred to an incubation container. The RBC unit is incubated at room temperature (20-25°C) for up to 18 hours. At the end of the incubation period the RBC unit is centrifuged, the supernatant is expressed, and the RBC unit is stored in fresh additive solution.

were those donated by the subject recipient. The identity of each returned unit was confirmed by comparison, and verification of the RBC extended phenotype using a panel of RBC major and minor antigens comparing preshipment samples to postshipment samples.

Chromium-51–labeled RBC 24-hour recovery

Samples for bacterial culture were taken from each unit at least 5 days before the planned reinfusion date. On Day 35 of Treatment Periods 1 and 2, the day of reinfusion, each study RBC unit (either test or control) was inspected for signs of hemolysis, clotting, or discoloration, and the bacterial culture was confirmed negative. The unit was cross-matched against a fresh subject blood sample. The unit was mixed by hand and an aliquot of the RBCs was collected from the unit and labeled with 10 to 20 μCi of ^{51}Cr using standard techniques.[28] The labeling agent, sodium 51-chromate, was mixed aseptically with the aliquot of RBCs at room temperature for 30 minutes followed by a wash step to remove any unincorporated label. An aliquot of the final volume was reserved for assay as a standard. Also on Day 35 of Treatment Periods 1 and 2, a fresh sample of heparinized blood (approx. 10 mL) was collected from the subject and treated with a sterile tin pyrophosphate solution, containing approximately 2.0 μg of tin. After a 5-minute incubation at room temperature, the autologous RBCs were washed with 2 volumes of cold saline; 20 μCi of technetium-99m (^{99m}Tc) pertechnetate was then added and incubated at room temperature for 10 minutes. After an additional cold saline wash, approximately 4 mL of the labeled RBCs was drawn up into a syringe. The ^{99m}Tc-labeled RBCs were carefully added to the ^{51}Cr-labeled RBCs and the two populations of labeled RBCs were infused for assessment of subject blood volume and RBC recovery and survival, respectively. The amount of RBCs and the amount of radioactivity infused were determined based upon the methods of Moroff and colleagues[28] and the International Committee for Standardization in Hematology.[29]

The infusion of the radiolabeled aliquot and subsequent sampling was carried out according to study site standard operating procedures and as described by Moroff and colleagues.[28] Briefly, after infusion of approximately 10 mL of stored ^{51}Cr RBCs mixed with freshly collected ^{99m}Tc-labeled RBCs, seven blood samples were drawn over the first 25 to 30 minutes after completion of the infusions and one at 24 hours (Day 36). Additional blood samples for ^{51}Cr activity assessment to measure life span were collected at 48 hours (Day 37), 72 hours (Day 38), and 7 days (Day 42) postinfusion and then weekly through 35 days postinfusion (Days 49, 56, 63, and 70). The radioactivity of the samples was counted in a gamma counter to determine ^{51}Cr- and ^{99m}Tc-specific activity. The measurement of ^{51}Cr provided an assessment of RBC recovery, and the measurement of ^{99m}Tc was used to measure blood volume.[28] All ^{51}Cr specimens used for survival analysis were counted at the same time and corrected for decay.

RBC hematologic and chemical analysis

An aliquot was removed from the RBC units immediately after collection (Day 0 of Treatment Periods 1 and 2) and on the day of infusion (Day 35 of Treatment Periods 1 and 2) using closed-system sampling methods. These samples were assessed for RBC physiologic function measured by in vitro variables (pH at 37°C, free Hb, adenosine 5′-triphosphate [ATP], 2,3-diphosphoglycerate [2,3-DPG]), extracellular potassium, extracellular glucose, extracellular lactate, packed cell volume, and mean corpuscular Hb concentration (MCHC) as previously described.[30] In addition, a complete blood count with differential was obtained on all study units on Days 0 and 35.

Immunohematologic analysis

Sera from the subjects obtained before infusion on Day 35 of Treatment Periods 1 and 2 were tested for antibodies to autologous control and autologous test RBCs using a commercial anti-human immunoglobulin G gel card assay (Ortho Clinical Diagnostics, Raritan, NJ). In addition, test and control sera, collected and frozen on Days 49 and 70 of each period, were screened against three individual units of S-303–treated and untreated allogeneic RBCs to detect antibodies to S-303 RBCs. The allogeneic RBCs (with the phenotype O, Rh cde, K–) were prepared as conventional RBCs and as S-303–treated RBCs. Allogeneic RBCs were utilized to test the serum samples from Day 49 and Day 70. These assays were conducted at a central reference laboratory (American Red Cross Blood Services, Pomona, CA).

Calculations and statistical analysis

RBC life span was estimated by dividing the ^{51}Cr raw counts (adjusted for Hct) for time points greater than 24 hours (N_t) by the counts at time zero (N_0). The estimate of the percentage of surviving cells was corrected for the loss of ^{51}Cr label over time by using an elution multiplicative factor (emf).[31] Linear and exponential functions were used to fit the data distribution and a weighted mean approach was used to derive a single estimate of T_{50} and mean life span. The estimates of mean life span and T_{50} from each model (linear and exponential) were pooled as follows:

$$\text{Mean lifespan (weighted)} = \frac{(MSE_{exponential} \times \hat{\mu}_{linear} + MSE_{linear} \times \hat{\mu}_{exponential})}{(MSE_{exponential} + MSE_{linear})} \text{ and}$$

$$T_{50}\text{ (weighted)} = \frac{(MSE_{exponential} \times \hat{m}_{linear} + MSE_{linear} \times \hat{m}_{exponential})}{(MSE_{exponential} + MSE_{linear})}$$

where MSE stands for the mean square error and $\hat{\mu}$ and $\hat{m}$ represent the means for each of the models (linear or exponential).

The AUC was determined by the trapezoid rule[32] where the survival curve is divided into a series of trapezoids (between time points), and the area of each trapezoid is calculated by the product of the width and mean height. The overall AUC is the sum of the trapezoidal areas.

For RBC recovery analysis, carryover effect was deemed insignificant ($p \geq 0.10$) so that a period-adjusted t test was used to test for treatment difference in mean recovery values. Unless otherwise stated, statistical analyses were performed using an analysis of variance (ANOVA) model with terms for sequence, period, treatment, and subject nested within sequence, where subject nested within sequence was a random effect, while the others were fixed effects, and except when stated otherwise, statistical tests were conducted using a two-sided alternative hypothesis at a significance level of 0.05. The one-sided 95% confidence interval (CI) for proportion of patients with a recovery of at least 75% was determined via the Clopper-Pearson method.

RESULTS

Demographics and baseline subject characteristics

Table 1 displays the demographic characteristics of the study subjects including ABO/Rh blood group. A total of 27 subjects, 11 males and 16 females, ranging in age from 25 to 66 years, met entry criteria; and 26 completed both phases of the in vivo recovery and survival studies. Direct antiglobulin tests and antibody screen were negative for all subjects enrolled at screening.

TABLE 1. Subject and immunohematologic demographics at screening (indirect antiglobulin test)*

Sex	
Male	11 (40.7)
Female	16 (59.3)
Blood group	
A	9 (33.3)
B	7 (25.9)
AB	1 (3.7)
O	10 (37.0)
Rh	
Negative	3 (11.1)
Positive	24 (88.9)

* Data are reported as number (%).

RBC content and prestorage hemolysis of S-303–treated units

S-303–treated RBC products, immediately after processing and before storage, had a net weight of 297 ± 10 g, with a Hct of 59 ± 1.8% and a Hb concentration of 18.8 ± 0.8 g/dL corresponding to a total Hb content of 53.1 ± 3.6 g. The mean prestorage hemolysis level was 0.1 ± 0.0%.

Twenty-four–hour posttransfusion recovery

The mean posttransfusion recoveries were 88.0 and 90.1% for test and control RBCs, respectively (Table 2). These means were greater than the Food and Drug Administration (FDA) guidance for a minimum mean recovery of 75%. The SDs for recovery in test and control were 8.50 and 6.88%, respectively, consistent with the FDA criteria of a maximum 9%. There was one subject in each treatment group with recovery less than 75%. The lower bound of the CI for the proportion of subjects with a recovery of 75% or greater exceeded the minimum of 70%. The lower confidence bounds for test and control were both 83.6%. The modest difference in mean 24-hour postinfusion recovery of test and control RBCs was not significant.

TABLE 2. 24-hour Cr/Tc posttransfusion recovery of RBCs (%)

	Test (n = 27)	Control (n = 27)	p value
Mean ± SD	88.0 ± 8.5	90.1 ± 6.9	0.31
Median	88.7	90.8	
Range	63.2-109.3	72.0-103.2	
Number of subjects with recovery < 75%	1	1	
95% LCB of one-sided CI for proportion of subjects with recovery ≥ 75%	83.6%	83.6%	
Mean difference (test – control)	–2.08		
Two-sided 95% CI of mean difference	–6.20 to 2.04		

LCB = lower confidence boundary.

RBC survival

The mean life span and T_{50} of RBCs was shorter compared to that of control RBCs (Table 3). The mean life spans (calculated by the weighted mean method) for test and control were 75 and 88 days, respectively. The mean difference in mean life span between test and control was –14 days ($p = 0.0008$). The mean T_{50} (calculated by the weighted mean method) for test and control were 33 and 40 days, respectively. Similar to the results obtained for mean life span, the mean median life span was significantly reduced in test compared to control for both sites combined ($p < 0.0001$). The mean AUCs for surviving cells, for test and control groups, were 23 and 24%, respectively ($p = 0.0124$).

Poststorage biochemical variables

In vitro variables indicative of RBC quality were analyzed for test and control RBCs (Table 4). On Day 35, mean hemolysis levels were 0.24 and

TABLE 3. Mean life span and T_{50} of RBCs

	Test (n = 26)	Control (n = 26)	Mean difference (test – control)	Two-sided 95% CI of mean difference	p value
Mean (days)	74.6	88.3	−13.73	−21.12 to −6.36	0.0008
T_{50} (days)	32.7	39.5	−6.77	−9.57 to −3.98	<0.0001
AUC (% × days)	22.5	23.9	−1.38	−2.44 to −0.33	0.0124

TABLE 4. RBC characteristics (mean ± SD)

Characteristic	Control (Day 35)	Test (Day 35)	p value
ATP (µmol/g Hb)	3.59 ± 0.808	3.34 ± 0.799	0.0028
Extracellular potassium (mmol/L)	52.7 ± 6.48	39.5 ± 3.56	<0.0001
Hemolysis (%)	0.22 ± 0.133	0.24 ± 0.132	0.44
Free Hb (mg/dL)	106.9 ± 64.95	104.43 ± 55.8	0.2628
Extracellular glucose (mmol/L)	14.6 ± 2.11	28.7 ± 5.45	<0.0001
Extracellular lactate (mmol/L)	28.8 ± 3.44	18.1 ± 2.72	<0.0001
pH at 37°C	6.483 ± 0.122	6.404 ± 0.039	0.0078
2,3-DPG (µmol/g Hb)	0.29 ± 0.280	0.12 ± 0.137	0.8202
MCHC (g/dL)	32.5 ± 1.96	33.9 ± 1.68	<0.0001
Spun Hct (%)	59.7 ± 2.4	62.4 ± 3.6	<0.0001

TABLE 5. Summary of AEs by subject

AE type	Test (n = 27)	Control (n = 27)	Overall (n = 27)	p value
Any AE	8	3	11	0.23
Mild (Grade 1)	5	3	8	
Moderate (Grade 2)	2	0	2	
Severe (Grade 3)	1	0	1	
Potentially life-threatening (Grade 4)	0	0	0	
Possibly or probably related AE	4	3	7	1.0
After donation and before infusion	1	1	2	
Postinfusion	3	2	5	
AE leading to study discontinuation	0	0	0	
Serious AE	1	0	1	

0.22% for test and control, respectively, with a maximum level of hemolysis at of 0.61% in test and 0.66% in control RBCs.

Biochemical variables measured on Day 35 showed glucose levels of 28.7 and 14.6 (mmol/L), for test and control, respectively, while mean lactate values increased to 18.1 and 28.8 (mmol/L) and pH dropped to 6.40 and 6.48 for test and control RBCs, respectively, suggesting increased glucose catabolism in S-303–treated RBCs. Mean potassium values on Day 35 were significantly lower in the test group as a consequence of the exchange protocol. The exchange protocol did not significantly modify the levels of 2,3-DPG or ATP immediately after processing. On Day 35, the mean levels of ATP were 3.34 and 3.6 µmol/g Hb for test and control RBCs, respectively, and, as expected, the intracellular concentration of 2,3-DPG was completely depleted by Day 35 of storage.

Immunohematologic analysis

In the context of a small-dose infusion, the results of the crossmatch assays on subjects' sera were negative for all subjects before infusion on Day 35. After the infusion, on Days 49 and 70 sera from all subjects were collected and were tested against allogeneic RBCs. All crossmatch analyses were negative, except for one subject with a weakly positive reaction (1+) for only one out of three test RBCs, on Day 49 after infusion of control RBCs in Period 2, suggesting that this reactivity was not likely to be specific for S-303–treated RBCs. The subject's Day 70 serum sample for the interval after infusion of S-303–treated RBCs was negative. Because there were no positive crossmatch tests to S-303 RBCs after RBC exposure, no further testing was done.

AEs

Overall, 12 AEs were recorded for 11 subjects. During the 24 hours after infusion, eight subjects experienced AEs after test RBCs and two subjects experienced AEs after the control RBCs ($p = 0.2266$, Table 5). One severe AE was due to hospitalization for severe knee inflammation 27 days after the infusion of test RBC infusion in Treatment Period 2. However, this latter event was consistent with an existing condition and was considered unlikely to be related to the study treatment. Three moderate AEs were reported by two subjects within 44 days after infusion of test RBCs. These events included nonhemorrhagic, papular rash in extremities of unknown etiology after receiving the RBC infusion in one subject and moderate stomach pain and diarrhea after receiving the RBC infusion in another subject. Both events were judged as unlikely related to study treatment by the local investigator: mild AEs within 24 hours after S-303–treated RBC infusion included mild, transient, decreased diastolic blood pressure, hyperhydrosis, iron deficiency, and dizziness. Mild AEs within 24 hours after control RBC infusion included local skin reaction at infusion site and increased blood lactate dehydrogenase. The overall AE incidence was not different between the test and control group infusions ($p = 0.23$, McNemar's test) and not different for AE possibly or probably associated with treatment ($p = 1.0$).

DISCUSSION

S-303 is derived from a new class of frangible anchor-linked effectors with labile alkylating activity.[25] The first generation of the S-303 RBC treatment system demonstrated inactivation of a large spectrum of pathogens and WBCs[33,34] and was evaluated in a series of six clinical studies. Four of these studies were radiolabel recovery studies in healthy subjects,[23,24] and two were Phase III studies in patients requiring therapeutic RBC transfusion support.[25,26] The Phase III clinical study of chronic RBC transfusion was terminated before completion when antibodies to S-303–treated RBCs were detected in two patients requiring chronic RBC transfusion support.[27] Low-titer antibodies against acridine, present on the membrane of the S-303–treated RBCs were detected by positive crossmatch reactions of the patient sera to S-303–treated RBCs. Antibody titers declined to undetectable levels in both patients during the follow-up period, and were shown to be unable to react against S-303–treated RBCs which were prepared with a modified protocol similar to the one presented in this article.[35] A second clinical trial of patients receiving transfusion of S-303–treated RBCs during cardiac surgery showed an identical frequency of crossmatch reactivity to S-303 RBCs (2 of 74 patients in test and control).[25]

A second generation of the S-303 treatment process, which was found to reduce the potential for immune reactions and maintain acceptable RBC in vitro quality throughout 35 days of storage, was tested in this Phase I clinical trial. The primary objective of this study was to evaluate the posttransfusion viability of autologous RBCs prepared using the second generation S-303 pathogen inactivation process and stored for 35 days. The study utilized a crossover design, which has the major advantage of allowing intrasubject comparison between treatments by minimizing the intersubject variability from the comparison. The primary endpoint was the posttransfusion recovery 24 hours after infusion of test and control RBCs. This primary endpoint was selected because 24-hour posttransfusion recovery represents the most commonly used criterion indicative of RBC transfusion efficacy. The 24-hour recovery was not significantly different between test and control RBCs. The 24-hour recovery for test RBCs was 88.0 ± 8.5% and that for control RBCs was 90.1 ± 6.88%. These results fall within the FDA guidance criteria defining the quality of RBC components based on 24-hour posttransfusion RBC recovery with respect to both the absolute proportional recovery and the maximum allowed variability in the 24-hour recovery measurements.

Mean life span, T_{50}, and AUC were evaluated as additional measures to characterize the viability of S-303–treated RBCs. These variables were assessed by ANOVA statistical analysis for a two-period crossover study design and by clinical relevance based on the criterion of bioequivalence applied to other biologic variables defined by a biologic variable within approximately 20% of its reference value.[36] Approximately 1% of RBCs (senescent RBCs) are normally cleared from the circulation each day and endogenous RBCs survive for approximately 120 days.[31] The model used to calculate the clearance of ^{51}Cr-transfused RBCs affects the measurement of RBC T_{50} and life span.[37] Linear clearance is thought to be most consistent with senescence, that is, the oldest RBCs preferentially leaving the circulation. Exponential clearance is thought to reflect clearance by a random process, perhaps due to physical or metabolic changes that affect viability.[38] Following the International Committee for Standardization in Hematology recommendations,[29] we modeled RBC survival through both a linear and an exponential mathematical function and used a weighted mean model when neither the exponential nor linear regressions demonstrated superior fit.[29] The mean test and control RBC T_{50} values were 32.7 and 39.5 days, respectively ($p < 0.0001$). For mean AUC, test RBCs were 22.5%, while control RBCs were 23.9% of cells surviving ($p = 0.0124$). The mean life span for test RBCs was 74.6 days, while that for control was 88.3 days ($p = 0.0008$). Of notice, the mean life span results are probably subject to a higher variation due to the intrinsic limitation of ^{51}Cr survival analysis which uses data extrapolation to calculate the axis intercept. For additional accuracy of RBC survival, we are planning further determinations of long-term RBC survival in the context of allogeneic transfusions of biotinylated RBCs. Despite these statistical differences, it is of note that all these indicators of RBC viability fall within the general guideline of 20% variation from the control consistent with the criterion of bioequivalence. The mean T_{50} falls within the published reference range of 28 to 35 days for conventional autologous RBC infusions.[39]

In addition to in vivo measures of viability, a series of in vitro variables were used to assess the quality of S-303 RBCs stored for 35 days. Minimal hemolysis, measured by plasma free Hb, is an index of RBC quality. On Day 35 all RBC units (both test and control) had hemolysis levels of less than 1%. The maximum hemolysis on Day 35 was 0.61% in test and 0.66% in control RBC components. Mean hemolysis on Day 35 was not statistically different between test and control RBCs (0.24% vs. 0.22%, $p = 0.44$).

ATP is an important reflection of RBC glycolytic metabolism and correlates with posttransfusion RBC recovery. By Day 35 of storage, the mean values of ATP for both test and control were well above the threshold value of 2 µmol/g Hb, which is considered acceptable for transfusion[40] and the marginal decrease in ATP concentration of test RBCs was not associated with diminished 24-hour recovery or increased hemolysis.

To assess RBC hydration, MCHC was measured using an automated hematology analyzer. This is an important

variable because RBCs with altered hydration status may exhibit diminished life span. For freshly drawn RBCs, the MCHC reference value is 34 g/dL, with 2 SDs lower being 31 g/dL.[39] For control RBCs, the Day 35 MCHC was 32.5 g/dL and that for test was 33.9 g/dL ($p < 0.0001$). Both of these values are within the reference range for fresh RBCs. Overall, these in vitro data indicate that RBCs prepared using the S-303 treatment process remain viable and metabolically suitable for transfusion through 35 days of storage. These data are also consistent with the 24-hour posttransfusion recovery results and with the three indicators of RBC life span indicating bioequivalence between test and control RBCs.

AEs were recorded for 24 hours after autologous blood donation and for 24 hours after each study transfusion. No AE led to study discontinuation and no significant AE was found to have a temporal or causal relationship to the infusion of test RBCs. There were eight AEs in the test group and three AEs in the control group ($p = 0.23$). Most AEs were mild to moderate with the exception of one severe AE in the test period that was considered not related to the infusion of S-303–treated RBCs. Because of the small-volume exposure to the test article and specific design to define the in vivo RBC recovery, this study had limited power to assess safety events. Despite that, we did not observe any significant AEs with a temporal or causal relationship to the infusion of S-303–treated RBCs.

Two patients in the RBC chronic transfusion trial with the first-generation S-303 process developed antibodies to S-303–treated RBCs. The immunoreactivity observed was due to the acridine moiety of the S-303 molecule and the lack of involvement of the RBC surface in this reactivity was confirmed by complete inhibition using acridine alone, consistent with the absence of neoantigens on the RBC surface. These antibodies did not induce detectable hemolysis in vivo in either of the two patients. In addition, a monocyte monolayer assay showed no increased RBC phagocytosis in the presence of sera of these two patients.[26,27,35] This second-generation process was designed to reduce the level of acridine bound to the RBC surface, thus reducing the potential for immune response. This has been supported by in vivo studies using a rabbit model of chronic RBC transfusion.[41] In these studies, chronic transfusion of RBCs with high levels of acridine (similar to the first-generation process) elicited an acridine-specific antibody response, whereas transfusion of RBCs with low levels of acridine (similar to the second generation process) did not.

In the context of infusion of small dose of S-303–treated RBCs, there was no evidence of immune response to 35-day-stored RBCs prepared using the S-303 exchange treatment process. S-303–treated RBC antibodies were not detected in any of the subjects' specimens when followed for up to 70 days postinfusion. Although the overall amount of infused RBCs was small, the absence of detectable antibody formation supports progression to studies of repeated transfusions of full units for recognized therapeutic indications.

In summary, in this Phase I two-period crossover study conducted in healthy volunteers, the 24-hour post-transfusion recovery of RBCs prepared using the S-303 pathogen inactivation process was not significantly different than the recovery of control RBCs and complied with the FDA guidance criteria for 24-hour RBC recovery and hemolysis at the end of the storage period, with no significant difference between test and control RBCs. RBC survival assessed through three different variables fell within 20% of control RBCs, indicating bioequivalence of RBC viability. There were no significant imbalances between test and control RBCs with respect to safety. Pathogen-inactivated RBCs produced using the S-303 treatment process show broad bioequivalence with control RBCs and the product appears suitable for advanced clinical development.

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CONFLICT OF INTEREST

JAC, NR, PGP, DNW, LJD, LH, ZMS, and AS received research funding from Cerus Corp. LJD is a consultant for Cerus Corp. AE, MP, AN, CS, NM, WR, and LC are employees of Cerus Corp.

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B　個別症例報告概要

○　総括一覧表
○　報告リスト

個別症例報告のまとめ方について

個別症例報告が添付されているもののうち、個別症例報告の重複を除いたものを一覧表の後に添付した（国内症例については、資料３において集積報告を行っているため、添付していない）。

別紙様式第4

感染症発生症例一覧

	番号	感染症の種類		発生国	性別	年齢	発現時期	転帰	出典	区分	備考
		器官別大分類	基本語								
第18回	1	感染症および寄生虫症	B型肝炎	米国	男	8歳	2011/12/12	不明	症例報告	外国製品	識別番号3-11000046 報告日:2011年12月27日
	1	臨床検査	血中免疫グロブリンM増加	米国	男	8歳	2011/12/12	不明	症例報告	外国製品	識別番号3-11000046 報告日:2011年12月27日

血対課ID	受理日	番号	報告者名	一般名	生物由来成分名	原材料名	原産国	含有区分	文献	症例	適正措置報告
120036	24-Apr-12	120108	CSLベーリング	乾燥pH4処理人免疫グロブリン	人免疫グロブリンG	ヒト血液	ドイツ	有効成分	あり	あり	なし
120037	24-Apr-12	120109	CSLベーリング	乾燥pH4処理人免疫グロブリン	ペプシン	ブタ胃粘膜	米国	製造工程	なし	あり	なし

別紙様式第４

感染症発生症例一覧

	番号	感染症の種類		発現国	性別	年齢（歳）	発現時期（年/月/日）	転帰	出典	区分	備考			
		器官別大分類	基本語								識別番号	報告日	MedDRA (Ver.)	
第18回	18 -1	臨床検査	C型肝炎抗体陽性	米国	男性	57	2012/2/29	未回復	試験からの報告	外国製品	12000001	2012/6/4	15.0	2012年4月27日の初回報告後に追加報告をおこなったため、最新情報に更新した。報告回数：2回
第18回	18 -2	感染症および寄生虫症	C型肝炎	米国	男性	43	2011/12/26	未回復	自発報告	外国製品	11000048	2012/3/1	14.1	2012年1月26日の初回報告後に追加報告をおこなったため、最新情報に更新した。報告回数：2回
第18回	18 -3	臨床検査	B型肝炎コア抗体陽性	米国	女性	4	不明	不明	自発報告	外国製品	11000049	2012/2/6	14.1	
第18回	18 -3	臨床検査	B型肝炎e抗体陽性	米国	女性	4	不明	不明	自発報告	外国製品	11000049	2012/2/6	14.1	
第18回	18 -4	臨床検査	検査結果偽陽性	米国	男性	40	不明	不明	自発報告	外国製品	11000045	2011/12/27	14.1	

血対課ID	受理日	番号	報告者名	一般名	生物由来成分名	原材料名	原産国	含有区分	文献	症例	適正措置報告
120061	22-Jun-12	120216	バクスター	乾燥イオン交換樹脂処理人免疫グロブリン	人免疫グロブリンG	人血漿	米国	有効成分	なし	あり	なし
120062	22-Jun-12	120217	バクスター	乾燥イオン交換樹脂処理人免疫グロブリン	人血清アルブミン	人血漿	米国	添加物	なし	あり	なし

追加資料

献血後の水分補給と休憩の徹底について

血液対策課

1. 背 景

○ 献血後に交通事故に遭遇し死亡される事例が発生。

2. 献血時の現状

○ 献血者に対して献血前に、献血後の水分補給と休憩の必要性などの注意点について説明資料を手渡して、献血終了時までに一読してもらっている。

○ 献血終了後、水分補給と休憩の必要性などについてあらためて説明している。

○ 献血終了後、接遇担当者が献血者に対して水分補給と休憩を促している。

3. 今後の対応方針

○ 献血者に対し、献血後の水分補給と休憩の必要性などについての周知を徹底する。