patients could be infected by TTV at least six times per year or 60 times during the first 10 years of blood transfusion therapy [Al Moslih et al., 2004]. Thus, patients with a 10-year transfusion history could have been infected or re-infected by all genotypes existing in the UAE. In addition, the extent of virus replication in thalassemia patients may be higher due to the large viral inocula injected directly into the blood stream through transfusion. This is obviously different from the small amount of virus acquired through infection via the oral route in normal blood donors.

It was not possible to conclude that TTV infection enhances the severity of liver disease in HCV infected patients because very few patients infected with HCV alone were available for comparison with patients co-infected with TTV and HCV. It is obvious that HCV plays a more important role than TTV in the development of severe liver disease.

It is well known that TTV infections are persistent. Consequently, the presence of TTV-negative thalassemia patients was unexpected. We do not yet have an explanation for this observation. Perhaps TTV host dependent genetic factors play an important role in determining the resistance or outcome of TTV infection among patients.

Follow-up studies of TTV infection and clearance in TTV-negative and TTV-positive thalassemia patients will eventually provide clues to understanding the natural history and pathogenesis of TTV. Of equal importance, a thorough understanding of the immune response to TTV infection, including viral persistence, quasipecies evolution, and viral immune escape, is needed to characterize the disease causing potential of this new group of viruses.

ACKNOWLEDGMENTS

Y.-W. Hu, M.I. Al-Moslih and E.G. Brown designed the research and wrote the manuscript H.P., S.U. and S.K. performed the research O.-L.Y. and J.W. analyzed the data M.T.A. provided valuable samples.

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別紙様式第2-1

No. 32

医薬品 研究報告 調査報告書

識別番号・報告回数			報告日	第一報入手日	新医薬品等の区分	機構処理欄
				2008. 2. 22	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	ProMED 20080218.0645, 2008 Feb 18. 情報源:[1]G1 Globo.com, 2008 Feb 13. [2]Milenio.com, 2008 Feb 17.	公表国	[1]ブラジル[2]パラグアイ
販売名(企業名)	新鮮凍結血漿「日赤」(日本赤十字社) 新鮮凍結血漿-LR「日赤」(日本赤十字社)				

研究報告の概要 119

○南米における黄熱のアウトブレイク
[1]ブラジル
2008年1月21日、32歳の男性が黄熱のため死亡した。これは、ブラジルで発生した15人目の黄熱死亡患者である。保健当局の発表によると、この男性は2月13日に感染が確認されており、首都ブラジリア近郊のソブランディーノの病院で死亡した。ブラジリアで感染したと見られている。また、Mato Grossoでも1名の感染と死亡が確認された。
[2]パラグアイ
保健当局は2月16日に、首都アスンシオンの病院で集中治療を受けていた39歳の女性が死亡したと発表した。パラグアイではこれまでに、少なくとも6名が黄熱によって死亡した。多くの市民がワクチン投与を求めて病院に殺到している。政府は944,000人分のワクチンをブラジルから輸入した。その大半はブラジル政府から寄付されたものである。ドゥアルテ大統領は15日、黄熱感染対応のため非常事態宣言を発令した。

報告企業の意見	今後の対応
南米で黄熱の流行が拡大し、パラグアイで6名、ブラジルで15名の黄熱死亡患者が発生したの報告である。	日本赤十字社は、輸血感染症対策として献血時に海外渡航歴の有無を確認し、帰国(入国)後4週間は献血不適としている。今後も引き続き情報の収集に努める。

使用上の注意記載状況・その他参考事項等

新鮮凍結血漿「日赤」
新鮮凍結血漿-LR「日赤」

血液を介するウイルス、細菌、原虫等の感染
vCJD等の伝播のリスク

(7)

Back

Archive Number 20080218.0645
Published Date 18-FEB-2008
Subject PRO/AH/EDR> Yellow fever - South America (02): Paraguay, Brazil

YELLOW FEVER - SOUTH AMERICA (02): PARAGUAY, BRAZIL
****
A ProMED-mail post
<http://www.promedmail.org>
ProMED-mail is a program of the
International Society for Infectious Diseases
<http://www.isid.org>

[1] Brazil
[2] Paraguay

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[1] Brazil
Date: Wed 13 Feb 2008
Source: G1 Globo.com [in Portuguese, trans. Mod. TY, edited]
<http://g1.globo.com/Noticias/Brasil/0,,MUL297999-5598,00.html>

A 32-year-old man died in Brazilia of yellow fever (YF) on 21 Jan 2008. With this death, the number of deaths in the country due to this disease has increased to 15.

The Secretary of Health of the Federal District (DF) confirmed this additional death from yellow fever on Wednesday [13 Feb 2008]. The man died at the hospital in Sobradinho, a satellite city of Brasilia. The report confirming the cause of death was issued this past Wednesday [13 Feb 2008].

According to the Ministry of Health, the likely location of infection of the man was in the Federal District. That contradicts what the health authorities in Brasilia have previously expressed. According to them, prior to this announcement, the people who died of YF in the DF had all been infected in Goias [state]. Of the cases reported in the DF, 11 were confirmed, 3 are being investigated and 2 were discarded [based on] clinical [grounds] and laboratory [results].

Mato Grosso
The Ministry of Health, also confirmed on Wednesday [13 Feb 2008], the 1st YF case in Mato Grosso (MT). Laboratory tests performed by the Evandro Chagas Institute, in Para, indicated that a farmer from Novo Sao Joaquim, MT died of the disease.

According to the Ministry of Health, the state of Mato Grosso has 2 other suspected cases of the disease which are still under investigation.

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Communicated by:
ProMED-mail <promed@promedmail.org>

[This worrisome report indicated that the man who died of YF acquired his infection in a satellite city of the DF, suggesting possible urban transmission. ProMED-mail requests more information concerning the probable location of infection and the travel history of the above mentioned fatality (in the DF), in order to have a better idea if this was another sylvan (jungle or forest) YF case or was truly a case of urban YF virus transmission. The Mato Grosso death is very likely a sylvan YF case.

An interactive ProMED health map of Brazil showing the location of Goias and Mato Grosso states and the Federal District can be accessed at: <http://healthmap.org/promed?v=-10.8,-53.1,4>. - Mod.TY]]

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[2] Paraguay
Date: Sun 17 Feb 2008
Source: Milenio.com [in Spanish, trans. & summ. Mod. TY, edited]
<http://www.milenio.com:80/index.php/2008/02/17/194717/>

Health authorities reported this Sunday [17 Feb 2008] that a 39-year-old woman died Saturday night [16 Feb 2008], after a week of intensive therapy in a hospital in the capital [Asuncion].

At least 6 people have died in Paraguay as a result of the yellow fever (YF) outbreak which has the entire population on alert, and responding with a massive [influx going to] vaccination centers, the government announced. Thousands of citizens went to the health centers in the capital where massive vaccination is taking place.

This weekend, the country received 944 000 doses of [YF] vaccine from Brazil, of which 800 000 were donated by the government of the neighboring country and 144 000 were furnished by the Panamerican Health Organization.

Nicanor Duarte, the President of Paraguay, this past Friday [15 Feb 2008] declared a national state of emergency to address the YF outbreak, so that the [governmental] authorities can deal with this health emergency.

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Communicated by:
ProMED-PORT <promed@promedmail.org>

[Given the massive vaccination campaign in the capital city, it appears that the previous urban YF cases that were acquired there have generated considerable concern (panic?) on the part of both the government and the citizens. ProMED-mail would be interested to know if similar vaccination campaigns are being carried out in other areas of Paraguay. Brazil, which had embargoed the export of the YF vaccine produced there, has shown remarkable public health citizenship by providing vaccine to Paraguay in a very timely way, despite continuing YF cases in Brazil.

A map of Paraguay can be accessed at:
<http://www.lib.utexas.edu/maps/americas/paraguay_pol98.jpg>. - Mod.TY]

[see also:
Yellow fever - South America: Paraguay, Brazil 20080217.0627
Yellow fever - Paraguay (03): (San Pedro): corr. 20080209.0533
Yellow fever - Paraguay (03): (San Pedro) 20080208.0511
Yellow fever - Paraguay (02): (San Pedro) alert 20080206.0475
Yellow fever - Paraguay (San Pedro) 20080205.0467
Yellow fever, monkeys - Argentina (02): conf. 20080212.0568
Yellow fever - Brazil (10): 20080205.0461
Yellow fever, monkeys - Argentina: (Misiones), susp. 20080205.0459
Yellow fever - Brazil (09): 20080203.0439
Yellow fever - Brazil (08): 20080124.0293
Yellow fever - Brazil (07): 20080119.0240
Yellow fever - Brazil (06): 20080116.0203
Yellow fever - Brazil (05): conf. 20080115.0194
Yellow fever - Brazil (04): susp. 20080111.0147
Yellow fever - Brazil (03) 20080110.0139
Yellow fever - Brazil (02): alert 20080109.0107
Yellow fever - Brazil: (Goias) susp. 2007 20080105.0056
2007

Yellow fever, monkeys - Brazil: (Goias), susp., RFI corr. 20071231.4196
Yellow fever, monkeys - Brazil: (Goias, Fed. Distr.): conf. 20071229.4173
Yellow fever, human, monkey - Brazil, Bolivia: 2007 20071224.4126
Yellow fever, monkey - Brazil (PI): susp 20071222.4119
Yellow fever, monkeys - Brazil (Goias): susp., RFI 20071217.4052
Yellow fever, monkeys - Brazil (RS): alert 20070910.2979
Yellow fever, human, monkey - Brazil (MG): not 20070508.1486
Yellow fever - Brazil (GO): alert 20070424.1335

Yellow fever, human, monkey - Brazil (MG) 20070421.1304]
.....mpp/ty/mpp

別紙様式第2-1

No. 15

医薬品 研究報告 調査報告書

識別番号・報告回数			報告日	第一報入手日	新医薬品等の区分	機構処理欄
				2007. 11. 22	該当なし	

一般的名称	(製造承認書に記載なし)	研究報告の公表状況	Ziemann M, Krueger S, Maier AB, Unmack A, Goerg S, Hennig H. Transfusion. 2007 Nov;47(11):1972-83.	公表国 米国
販売名(企業名)	合成血「日赤」(日本赤十字社) 照射合成血「日赤」(日本赤十字社) 合成血-LR「日赤」(日本赤十字社) 照射合成血-LR「日赤」(日本赤十字社)			

研究報告の概要

○セロコンバージョンと関連した供血者血漿検体中のサイトメガロウイルスDNAの高頻度陽性

背景:ヒトサイトメガロウイルス(CMV)は、血液細胞に潜伏感染すると考えられている。免疫不全患者の輸血感染(TT-CMV)は、CMV-血清反応陰性成分または白血球除去成分を使用しても発現する。

試験デザインおよび方法:過去にCMV血清反応陰性で、初めて抗CMV IgG陽性を示した供血者82名、1年以上血清反応陽性である供血者598名、血清反応陰性供血者150名を対象として、血漿中のCMV DNA陽性率を検討した。本試験後半では、供血血液31,745に基づく供血血液全体のCMV DNA陽性率を評価した。

結果:CMV DNAは、新たに血清反応陽性となった供血者の血漿検体の44%に反復的に検出された(直近前回の血清反応陰性成分供血までの期間に応じて12%～62%の範囲)。継続的な血清反応陽性または血清反応陰性供血者はいずれも、CMV DNA陰性であった。セロコンバージョンに関連したCMV DNAの検出は、ネオプテリンの有意な増加、ALT増加、白血球数減少と関連付けられたが、これら代替マーカーの感度はわずか71%であった。CMV初感染供血者による血液製剤中のCMV DNAの全体的な陽性率は0.13%以上であった。

結論:白血球除去の実施にもかかわらず、新規血清反応陽性供血者のウイルス血症はTT-CMV残存リスクの重大原因であると考えられる。本試験ではウインドウ期が検出可能で、再燃は検出できなかったため、血清反応陰性供血者由来の白血球除去血液の輸血には、1年以上血清反応陽性である供血者由来白血球除去血液の輸血と比較して、TT-CMVの高いリスクが示される可能性が考えられた。

報告企業の意見	今後の対応
新規CMV血清反応陽性供血者は血漿中のCMV DNA陽性率が高く、白血球除去を実施していてもTT-CMV残存リスクの重大原因であると考えられるとの報告である。	CMV感染に関する新たな知見等について今後も情報の収集に努める。

使用上の注意記載状況・その他参考事項等

合成血「日赤」
照射合成血「日赤」
合成血-LR「日赤」
照射合成血-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

High prevalence of cytomegalovirus DNA in plasma samples of blood donors in connection with seroconversion

Malte Ziemann, Sabine Krueger, Andrea B. Maier, Alexander Unmack, Siegfried Goerg, and Holger Hennig

BACKGROUND: Human cytomegalovirus (CMV) is considered to latently infect blood cells. Transfusion-transmitted infection (TT-CMV) of immunocompromised patients occurs despite the use of CMV-seronegative or leukoreduced units.

STUDY DESIGN AND METHODS: The prevalence of CMV DNA in plasma was investigated in 82 blood donors who had previously been seronegative for CMV and showed anti-CMV immunoglobulin G for the first time, 598 blood donors who were seropositive for at least 1 year, and 150 seronegative blood donors. In a second part of the study, the overall prevalence of CMV DNA in blood donations was assessed based on 31,745 donations.

RESULTS: CMV DNA was repeatedly detected in plasma samples of 44 percent of newly seropositive donors (12%-62%, depending on the interval to the last seronegative donation). All steadily seropositive or seronegative donors were negative for the presence of CMV DNA. Detection of CMV DNA in connection with seroconversion was accompanied by significantly increased neopterin, increased alanine aminotransferase, and reduced white blood cell counts, but the sensitivity of these surrogate markers was only 71 percent. The overall prevalence of CMV DNA in blood products due to primary CMV infection of donors was at least 0.13 percent.

CONCLUSION: Viremia of newly seropositive donors may be an important reason for the residual risk of TT-CMV despite leukoreduction. Furthermore, transfusion of WBC-reduced blood components from seronegative donors could imply a greater risk of TT-CMV than transfusion of WBC-reduced blood from donors who have been seropositive for at least 1 year, because window-phase donations but no reactivation could be detected in this study.

Human cytomegalovirus (CMV) is an ubiquitous β-herpesvirus causing mostly asymptomatic or mild mononucleosislike infections in immunocompetent subjects[1] with a prevalence of between 40 and 100 percent in adult populations.[2] Contrarily, infection of immunocompromised patients with CMV is a significant cause of morbidity and mortality. Symptoms of CMV infection in these patients cover a broad range from direct manifestations of viral replication like fever, leukopenia, thrombocytopenia, hepatitis, enteritis, and pneumonia to indirect sequelae like an elevated risk for renal allograft rejection or an impaired cellular immune response.[3,4]

An important route of infection for risk groups like seronegative recipients of marrow transplants or newborns is assumed to be transmission of CMV by blood products from latently infected blood donors (so called transfusion-transmitted CMV infection [TT-CMV]). Even if the exact sites and mechanisms of latency still remain to be clarified, CMV DNA has repeatedly been found in peripheral blood white blood cells (WBCs) of healthy, CMV-seropositive individuals, especially in cells of the myeloid lineage.[5,6]

Consequently, leukodepletion of blood products and inventories of seronegative blood donors have been employed to reduce the rates of TT-CMV since the 1980s.[7,8] Even after implementation of these strategies, however,

ABBREVIATIONS: CRP = C-reactive protein; TT-CMV = transfusion-transmitted cytomegalovirus.

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TRANSFUSION 2007;47:1972-1983.

"break-through" infections persist with rates as high as 1 to 3 percent of transfused high-risk patients.[9-15] The impact of active CMV infection of donors (both primary and reactivated) with transmission of infectious virus as a reason for these break-through infections is discussed controversially.[16-18]

To our knowledge, infectious virus has never directly been detected in leukodepleted blood components. This may be due to the relatively poor sensitivity of commonly applied viral cultures or shell vial assays[19] even if more sensitive methods have been described recently.[20] CMV DNA in serum or plasma, on the other hand, is associated with active CMV infection[21] and used routinely for diagnosis and monitoring of CMV infections in risk groups like transplant recipients[22] or acquired immune deficiency syndrome (AIDS) patients.[23]

Drew and coworkers[17] found CMV DNA in the last seronegative sample of 1 of 192 donors (0.5%) and in the first seropositive sample of 2 donors (1.0%), both of whom were excluded from donation because of elevated alanine aminotransferase (ALT). This contrasts with the findings of Zanghellini and colleagues[24] who detected CMV DNA in plasma of 4 of 5 adolescents with CMV seroconversion, but so far no further study has addressed the prevalence of CMV DNA associated with seroconversion of donors.

Because the actual date of seroconversion can be any point between the last seronegative and the first seropositive donation, the prevalence of CMV DNA in the plasma of first-time seropositive donors would be expected to be higher if the interval since the last seronegative sample is relatively short. Nevertheless, there are no data about the correlation between interdonation interval and prevalence of CMV DNA in plasma of newly seroconverted donors or the variations in prevalences of CMV DNA between different donor collectives.

Therefore, we conducted a prospective study, grouping newly seropositive donors according to the interval since their last seronegative sample and measuring CMV DNA in plasma samples before and after seroconversion. Another objective of our study was to determine the sensitivity of surrogate markers for viral infections, like neopterin, ALT, or WBC count for the detection of CMV DNA in plasma in connection with seroconversion.

MATERIALS AND METHODS

Blood donors

Between August 2000 and June 2004, approximately 12,800 volunteer regular blood donors (47% female, 53% male) donated approximately 34,000 whole-blood donations per year (41% by female and 59% by male donors). They were between 18 and 67 years old and healthy and gave informed consent before the donation. Out of this blood donor collective, we investigated 82 well-defined CMV seroconversion cases, whereas the total number of CMV seroconversions during this period has not been determined. Donors were grouped according to the interval since the last seronegative sample, with intervals of less than 120, 120 to 729, and 730 days or more.

Additionally, 598 latently infected blood donors who had been seropositive for at least 1 year were included in this study, 148 of whom had been excluded from donation because of elevated ALT (more than 73 U/L or 112 U/L for female and male donors, respectively). Sampling dates from latently infected donors were distributed evenly throughout the year considering potential seasonal reactivations.[16] A total of 150 CMV-seronegative donors were tested for CMV DNA as controls.

In a further part of the study, all available samples from previously seronegative donors who were repeatedly reactive in the recombinant CMV immunoglobulin G (IgG) enzyme-linked immunosorbent assay (ELISA) between January and December 2006 were tested by polymerase chain reaction (PCR) to determine the minimum rate of CMV DNA–positive donations due to primary CMV infection of donors in our donor population.

Blood specimens

Whole-blood samples were collected in 5.5-mL tubes containing potassium-ethylenediaminetetraacetate (EDTA) at a concentration of 1.6 mg EDTA per milliliter of blood (Monovette, Sarstedt, Nümbrecht, Germany). Such samples were centrifuged at $3291 \times g$ for 4 minutes and EDTA plasma was separated within 24 hours. Plasma specimens were stored at 4 to 8°C for no longer than 72 hours or at less than –30°C until further processing.

Because of the impossibility of determining the actual seroconversion date, the date of the first seropositive sample from a previously seronegative donor was assumed to be the date of seroconversion.

Standard and control specimens

Human CMV quantitated viral DNA control, AD169 strain, Lot 110-018 (Advanced Biotechnologies Inc., Columbia, MD) was used to determine the detection limit of the CMV PCR (TaqMan, Applied Biosystems, Foster City, CA) described below and to quantify CMV DNA–positive samples. Lyophilized CMV DNA–positive cells from an external proficiency testing program (Instand e.V., Düsseldorf, Germany) were used as positive samples for the development and optimization of the TaqMan CMV PCR.

CMV serology

Anti-CMV screening was performed with an automated enzyme immunoassay to detect IgG antibodies against the autologous fusion proteins CG1 and CG2 (Biotest Anti-CMV recombinant IgG ELISA, Biotest AG, Dreieich,

Germany). Reactive samples were retested in duplicate and considered to be repeatedly reactive if at least one of the two repetitions also gave a positive result. In the first part of the study, repeatedly reactive samples were further confirmed by an automated ELISA with AD169-coated microparticles (AxSYM CMV IgG, Abbott GmbH, Wiesbaden, Germany).

Nucleic acid isolation

DNA from 1 mL of EDTA-plasma was prepared using the Extractor (NucliSens™, bioMérieux Deutschland GmbH, Nürtingen, Germany) according to the manufacturer's protocol for pooled plasma or serum samples up to 2.0 mL. To increase the nucleic acid yield, we added a first incubation step of samples together with the lysis buffer, which is based on guanidine thiocyanate at 60°C with horizontal shaking at 110 r.p.m. for 30 minutes. Total nucleic acids from 1 mL of plasma were eluted in 50 µL of elution buffer of which 20 µL was investigated in one PCR experiment to detect CMV DNA.

During the last part of the study between January and December 2006, DNA from 1 mL of EDTA-plasma was isolated with magnetic extraction reagents (NucliSens, bioMérieux, Boxtel, the Netherlands) according to the manufacturer's instructions.

TaqMan PCR

For amplification and simultaneous detection of PCR products, we developed a novel approach based on a quantitative PCR core kit (qPCR, Eurogentec, Seraing, Belgium) on a sequence detection system (ABI Prism 7700 SDS, Applied Biosystems). Primers and fluorogenic TaqMan probe for CMV DNA detection were chosen after comparative analysis of 68 sequences containing the glycoprotein B region of the CMV genome, which were available from the GenBank Nucleotide Database with computer software (OMIGA, Version 2.0, Oxford Molecular, Oxford, UK). In addition, we performed a nucleotide-nucleotide BLAST search via the Internet at http://www.ncbi.nlm.nih.gov/BLAST/ for the chosen oligonucleotides. For the forward primer, TaqMan probe, and reverse primer, we found 84, 80, and 84 hits, respectively, to CMV sequences that had been submitted to various databases. The sequence alignments ensured that the primers were homologous at the last 12 nucleotides at the 3′ end in all of the sequences and showed a maximum of one single-nucleotide polymorphism at the upper sequence. The TaqMan probe showed 100 percent homology or only one mismatch to all of the CMV hits. Seventeen non-CMV BLAST hits each to only one of the three CMV oligonucleotides ensured that no other organism could be detected with this method.

A sequence from the human C-reactive protein (CRP) gene, which was found to be detectable in human plasma was coamplified in each reaction as internal control.[25] The CMV probe was labeled with FAM as reporter and TAMRA as quencher and the CRP probe with VIC and TAMRA dyes. The CMV primers and probe were custom-synthesized by Eurogentec (Liege, Belgium), the CRP probe by Applied Biosystems (Weiterstadt, Germany), and the CRP primers by TIB Molbiol (Berlin, Germany). The sequences of all the oligonucleotides are provided in Table 1.

PCR experiments were carried out in special optical tubes (MicroAmp optical tubes/caps, PE Applied Biosystems, Foster City, CA) in a total volume of 50 µL. Concentrations of $MgCl_2$, CMV probe and primers were optimized by means of chessboard titrations. Final concentrations were 3.5 mmol per L for $MgCl_2$, 150 nmol per L for CMV forward primer, 300 nmol per L for the respective reverse primer, and 250 nmol per L for the CMV probe. The concentration of the CRP probe was 100 nmol per L, whereas those of the CRP primers were limited to 40 nmol per L each. Thermal cycler conditions were 10 minutes at 95°C followed by 40 cycles of 15 seconds at 95°C and 1 minute at 60°C. Threshold values were calculated as the upper 10-fold standard deviation (SD) of the background fluorescence signal measured over the baseline from Cycle 3 to Cycle 30. Results were interpreted as follows: a C_T of less than 40 is positive; a C_T of equal to 40 is negative.

To determine the 95 percent detection limit of the TaqMan CMV PCR, we investigated semilogarithmic dilutions of the CMV quantitated viral DNA control containing between 10^2 and $10^{-0.5}$ genome equivalents per µL (geq/µL) of CMV strain AD169. Twenty-eight samples of each concentration were processed in four consecutive TaqMan PCR procedures according to our protocol for

TABLE 1. Primer and probe sequences

Oligonucleotide	Sequence 5′→3′	Melting temperature (°C)
CMV		
Forward primer	CCCTCAAGTATGGAGATGTGGTG	59
TaqMan probe	FAM-AACACCACCAAGTACCCCTATCGCGTG-TAMRA	69
Reverse primer	AGCGAATAAGATCCGTACCCTG	58
CRP		
Forward primer	CCTGACCAGCCTCTCTCATGC	61
TaqMan probe	VIC-TTTGGCCAGACAGGTAAGGGCCACC-TAMRA	70
Reverse primer	TGCAGTCTTAGACCCCACCC	59

plasma samples. The 95 percent detection limit was calculated by means of probit analysis. Quantification of CMV DNA–positive samples was carried out by means of a standard curve derived from these validation experiments.

Diagnosis of CMV DNAemia

All samples were analyzed by TaqMan PCR in duplicate. Samples with invalid internal control or diverging results were retested twice. DNAemia was diagnosed by reproducibly positive results.

Surrogate markers for viral infections

As part of the routine blood donor screening, ALT levels were determined by the standard IFCC method at 37°C (GPT ALAT liquid IFCC, Medizintechnik Guder, Bad Oeynhausen, Germany) with an automated analyzer (COBAS Mira plus CC, Roche Diagnostics Instruments Center, Rotkreuz, Switzerland). ALT screening was mandatory in Germany until 2004 with limits for donor admission of no more than 73 and 112 U per L for female and male donors, respectively. WBC counts were measured with an automated hematology analyzer (Coulter Gen S, Beckman Coulter, Krefeld, Germany).

In a subgroup of 56 samples, the neopterin concentration was analyzed by an ELISA (neopterin ELISA RE59349, IBL Immuno Biological Laboratories, Hamburg, Germany). The cutoff value for elevated neopterin was set at 10 nmol per L representing the 98th percentile of a healthy asymptomatic population.[26]

Statistical analysis

Unless stated otherwise, means are calculated as arithmetic means ± SD. Confidence intervals (CIs) were calculated with a p value of 0.05. Differences between groups were examined with the U test. Calculations were assisted by database and statistical programs (Excel, Microsoft Corp., Redmond, WA; SPSS, SPSS Inc., Chicago, IL). The probability of appearance of CMV DNA in plasma of latently infected blood donors was calculated with the upper limits of $1 - \alpha$ confidence intervals of the binomical distribution for an α level of 0.05.

The sensitivity of surrogate markers for detection of CMV DNA–positive donations was calculated as the number of CMV DNA–positive donations with elevated markers related to the total number of CMV DNA–positive donations tested for this marker. For neopterin, for instance, this results in the formula

Sensitivity = (Number of CMV DNA–positive donations with elevated neopterin)/(Number of CMV DNA–positive donations tested for neopterin).

The percentage of patients potentially transfused with CMV DNA–positive blood components due to primary CMV infection of donors was calculated according to the formula

$$\%\text{Patients} = 100 \times [1 - (1 - p)^{\text{number of units transfused}}].$$

In this formula, p denotes the proportion of CMV DNA–positive donations related to all donations. Therefore, $(1 - p)$ is the probability of donations being negative for the presence of CMV DNA, and $(1 - p)^n$ is the probability of n units of blood from different donations all being negative for CMV DNA. So $1 - (1 - p)^n$ equals the probability of n units blood containing at least one CMV DNA–positive unit.

RESULTS

TaqMan PCR

Of 1055 plasma samples tested by TaqMan PCR, 1042 (98.8%) were clearly positive or negative, whereas only 13 (1.2%) showed ambiguous results. These were due to insufficient sample volume for repeated testing (6 samples) or equivocal results even of repeated testing (7 samples with 2 positive and 2 negative results each). All samples with ambiguous results were excluded from analysis.

The 95 percent detection limit of the TaqMan PCR was calculated to be 4.88 geq per PCR procedure (3.66-8.22 geq/PCR) with semilogarithmic dilutions of CMV quantitated viral DNA control (Table 2). For 1-mL plasma specimens, it would correspond to approximately 13.5 geq per mL, if an efficacy of DNA isolation of 90 percent is assumed. The mean CMV DNA concentration in positive samples was 166 geq per mL (SD, 395 geq/mL), with a maximum of approximately 3200 geq per mL.

CMV DNA in connection with seroconversion of blood donors

Eighty-two blood donors who were previously tested negative for the presence of CMV IgG antibodies at the

TABLE 2. Observed frequencies in TaqMan CMV PCR

Standard (geq/PCR procedure)	Number of subjects	Observed responses	Probit
100	28	28	1.000
31.6	28	28	1.000
10	28	28	0.999
3.16	28	21	0.784
1	28	15	0.384
0.316	27*	4	0.263

* One subject was excluded due to negative results for CRP DNA.

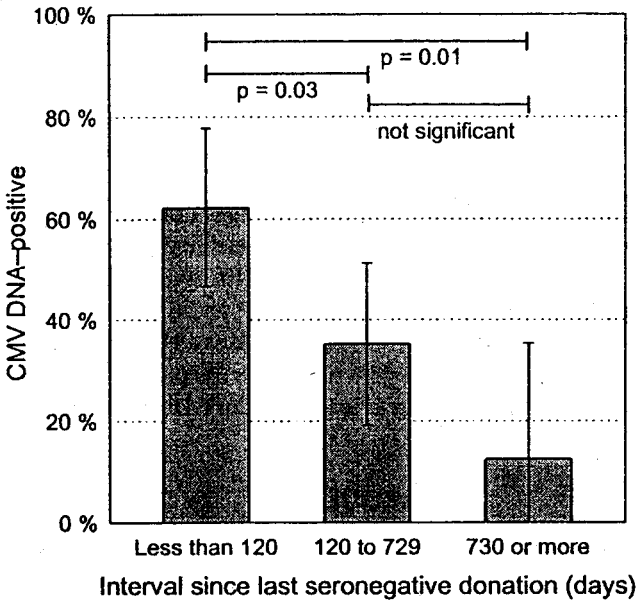

Fig. 1. Prevalence of CMV DNA in plasma of newly seropositive donors ($n = 79$) in dependence on the interval since the last seronegative sample. Prevalences are shown as percentages with 95 percent CI. The percentage of CMV DNA–positive donors was significantly greater after intervals of less than 120 days.

time of their last donation were repeatedly reactive in both anti-CMV assays and further investigated for the presence of CMV DNA. Thirty-six of 82 newly seropositive samples (44%) were repeatedly positive for the presence of CMV DNA by TaqMan PCR. The prevalence of CMV DNA was significantly higher if the interval since the last seronegative donation was less than 120 days ($p < 0.01$), whereas further differences between donors with longer intervals were not significant (Fig. 1).

In 68 (83%) of 82 seroconversion cases, we investigated the last CMV-seronegative donation before seroconversion. This way, we identified 2 reproducibly CMV DNA–positive window-phase donations (2.9%), 68 and 98 days before the first CMV-seropositive donation.

A second seropositive sample was available from 71 (87%) of 82 donors. Sixty-two of these samples (76%) were drawn within 1 year after the first seropositive sample. Samples from 4 donors were reproducibly CMV DNA–positive on Days 3, 5, 20, and 84 after the first seropositive sample, respectively (Table 3). Further plasma samples were available from only 2 of these donors. Both tested CMV DNA–negative on Day 97 and 207, respectively. So CMV DNA–negative samples were available from 59 of 82 donors (72%) within 1 year after the first seropositive sample, whereas no sample tested CMV DNA–positive 1 year or more after the first seropositive sample.

CMV DNA in latently infected and seronegative blood donors

All plasma samples of 150 seronegative and 450 latently infected donors who had been seropositive for at least 1 year were tested negative for the presence of CMV DNA. Additionally, 148 samples of latently infected donors with elevated ALT were available with mean ALT levels of 113 U per L (range, 76-906 U/L). These samples, too, all tested negative for the presence of CMV DNA. Based on the sample size, the proportion of CMV DNA–positive donors related to the total donor population (95% CI) was estimated to be less than 0.5 percent for latently infected donors and no more than 2 percent for latently infected donors with elevated ALT or seronegative donors.

Overall prevalence of CMV DNA in blood donations

In 2006, 102 previously seronegative donors tested repeatedly reactive in the recombinant IgG ELISA. This corresponds to an annual seroconversion rate of 0.8 percent relative to the total donor population.

Thirty-six donations from newly seropositive donors (41% of available samples) tested repeatedly positive for CMV DNA. Assuming the prevalence of CMV DNA in the first seropositive donation to be 41 percent for all 102 seroconversion cases results in a minimum rate of CMV DNA–positive units of 42 of 15,094 seropositive units (0.28%) or 42 of 31,745 units (0.13%), if the CMV serostatus is not taken into consideration (Fig. 2). These rates underestimate the actual prevalence of CMV DNA–positive units, because both window-phase donations and further seropositive donations containing CMV DNA have been ignored.

Surrogate markers for CMV DNAemia

The three common surrogate markers for subclinical viral infections, neopterin, ALT, and WBC count, have been tested in comparison to CMV DNA. Newly seropositive donors with CMV DNAemia had significantly higher neopterin and ALT values, as well as significantly lower WBC counts compared to newly seropositive donors without detectable CMV DNA in plasma. The best sensitivity was achieved by the neopterin ELISA, which detected 61 percent of CMV DNA–positive samples. The sensitivity of ALT was 42 percent if any values outside the normal range were considered. ALT values above the former German national limits for donor admission (>73 or 112 U/L for female and male donors, respectively) were detected in only 4 of 36 CMV DNA–positive donors and in no CMV DNA–negative donor. This equals a sensitivity of 11 percent.

WBC counts were slightly low in 6 of 36 CMV DNA–positive subjects (between 3.4×10^9 and 3.9×10^9/L),

TABLE 3. CMV DNA in plasma samples*

Donor status	Number of samples	CMV DNA		Excluded
		Positive	Negative	
Seronegative donors	150	0 (0%)	150 (100%)	0 (0%)
Donors with seroconversion				
Last seronegative sample	68	2† (3%)	64‡ (94%)	2§ (3%)
First seropositive sample	82	36 (44%)	43 (52%)	3‖ (4%)
Second seropositive sample	71	4¶ (6%)	66** (93%)	1†† (1%)
Donors who were seropositive for at least 1 year	450	0 (0%)	450 (100%)	0 (0%)
Donors with elevated ALT‡‡ who were seropositive for at least 1 year	148	0 (0%)	148 (100%)	0 (0%)

* Data are reported as number (%).
† Drawn 68 and 98 days before the first seropositive sample (median, 83 days).
‡ Drawn 15 to 1513 days before the first seropositive sample (median, 192 days).
§ One due to ambiguous PCR results and one due to insufficient sample volume for repeated testing after a positive PCR result. Drawn 35 and 105 days before the first seropositive sample (median, 70 days).
‖ One due to ambiguous PCR results and two due to insufficient sample volume for repeated testing after a positive PCR result.
¶ Drawn 3 to 84 days after the first seropositive sample (median, 12.5 days).
** Drawn 15 to 798 days after the first seropositive sample (median, 131 days). Fifty-seven of 66 samples (86%) were drawn after an interval of no more than 365 days.
†† Due to insufficient sample volume for repeated testing after a positive PCR result. Drawn 39 days after the first seropositive sample.
‡‡ 76 U/L or more.

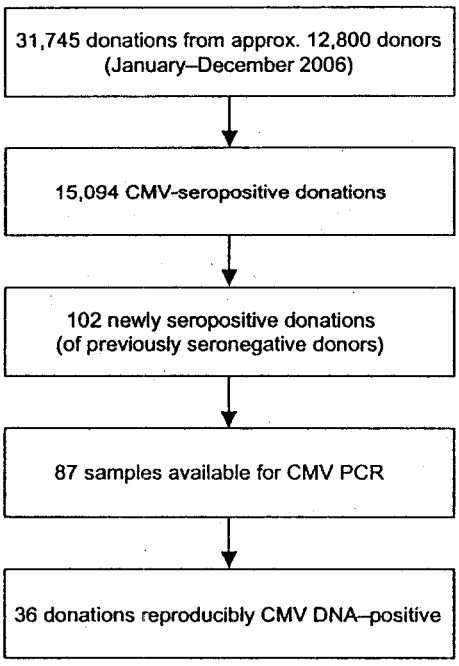

Fig. 2. Prevalence of CMV DNA in plasma of blood donors due to primary CMV-infections. Between January and December 2006, 36 out of 87 donations from newly seropositive donors contained CMV DNA (41%). 44 samples tested DNA-negative and 7 samples were excluded (5 because of ambiguous results and 2 due to insufficient sample volume for repeated testing after a positive PCR result). Assuming the prevalence of CMV DNA in the first seropositive donation to be 41 percent for all 102 seroconversion cases results in a minimum rate of CMV DNA–positive units of 42 out of 15,094 seropositive units (0.28%) or 42 out of 31,745 units (0.13%), if the CMV-serostatus is not considered.

resulting in a sensitivity of 17 percent. A combined screening with neopterin, ALT, and WBC counts would have a sensitivity of 71 percent, if all values outside the normal range are considered (Table 4).

DISCUSSION

The prevalence of CMV DNA in plasma of newly seropositive donors was 44 percent in our study. To our knowledge, the only other study examining CMV DNA in connection with seroconversion of blood donors is the study of Drew and colleagues[17] who detected CMV DNA in only 2 of 192 first-time seropositive donors (1%).

One possible reason for this marked difference is the lower limit of detection of the TaqMan PCR (~13.5 geq/mL) in comparison to the PCR applied by Drew and coworkers (400 geq/mL). In the first part of our study, only 4 of 82 newly seropositive donors (5%) had CMV DNA levels of 400 geq per mL or more in their first seropositive sample. Furthermore, the interval to the last seronegative donation is given as "8 weeks to years" in the study of Drew and coworkers without any mean or medium interval given. A high proportion of donors with long interdonation intervals could have led to a lower number of CMV DNA–positive donors, as the prevalence of CMV DNA in our study was significantly higher after short interdonation intervals. Even an influence of the different target sequence used by Drew and coworkers (pol instead of gB) cannot be excluded.

Zanghellini and colleagues[24] reported approximately 45 seronegative adolescents, who were screened for development of CMV antibodies at monthly intervals. They detected 6 seroconversion cases and tested plasma samples from 5 seroconverted adolescents by CMV PCR finding CMV DNA in samples from 4 subjects (80%). This

TABLE 4. Neopterin concentration, ALT level, and WBC count as surrogate markers for CMV DNA in plasma of newly seropositive donors

Surrogate marker	CMV DNA–positive donors	CMV DNA–negative donors
Neopterin concentration (n = 56)*	13.6 ± 9.1†	6.8 ± 2.8†
<10 nmol/L	11	24
≥10 nmol/L	17	4
Donor sensitivity (%)	61 (17/28)	
ALT level (n = 79)	43.9 ± 35.7†	23.2 ± 11.2†
≤30 or 40 U/L‡	21	37
>30 or 40 U/L‡	15	6
Donor sensitivity (%)	42 (15/36)	
WBC count (n = 78)§	5.2 ± 1.1†	6.3 ± 1.9†
$<4 \times 10^9$/L	6	3
Between 4 and 10×10^9/L	30	38
$>10 \times 10^9$/L	0	1
Donor sensitivity (%)	17 (6/36)	
Combined screening (n = 56)*		
All tests normal	8	21
Any test positive	20	7
Donor sensitivity (%)	71 (20/28)	

* Neopterin was measured in a subset of 59 donors, of whom 3 had ambiguous PCR results.
† $p = 0.001$, $p = 0.002$, and $p = 0.004$ for differences in neopterin concentrations, ALT levels, or WBC counts between DNA-positive and DNA-negative donors, respectively.
‡ Values for female and male donors, respectively.
§ WBC counts for one CMV DNA–negative donor are missing.

high prevalence of CMV DNA must be interpreted with caution owing to the low number of subjects studied, but it could be caused by the short screening interval, which would be in accordance with our results.

In a study of 420 blood donors conducted by Glock and coworkers,[18] CMV DNA in serum was detected solely in an IgG-positive donor with equivocal results for IgM, but not in 185 IgM-negative and IgG-positive donors. No information about the date of seroconversion is supplied by the authors, however.

Detection of CMV DNA in serum or plasma correlates well with presence of infectious virus in transplant recipients[21] and patients with AIDS.[23] Even if assays for detection of viable CMV with detection limits corresponding to 27 geq CMV DNA per mL have been described[20] most viral cultures or shell vial assays have relatively low sensitivities.[19,27] Therefore, detection of CMV DNA in plasma or serum is routinely used for diagnosis and monitoring of CMV infections in transplant recipients.[22] Consequently, CMV PCR has recently been suggested for screening of cord blood samples used for transplantation.[28]

The concerns of some authors[29] about lacking infectivity of CMV DNA–positive blood donations are based on a single study of three renal transplant recipients with active CMV infection showing CMV DNA in plasma to be highly fragmented.[30] But even the authors of this study conclude that, "It is beyond doubt that CMV DNA load measurements are important for prediction and diagnosis of CMV disease." Neutralizing antibodies against CMV could reduce the infectivity of seropositive CMV DNA–positive donations, but they are not expected to achieve complete neutralization as studies of convalescent sera showed neutralization capacities not exceeding 50 percent.[31]

Early studies of CMV DNA showed inconsistent results with some reports of high prevalences even in seronegative donors,[32-34] which could not be reproduced by validated PCR assays.[35] Therefore, the need for appropriate validation of PCR assays was stressed by Roback and associates.[36] Our TaqMan PCR was carefully designed to detect CMV genome with high sensitivity without cross-reaction with other organisms' DNA. Additionally, samples from all study populations were processed in arbitrary order, whereby CMV DNA was detected in connection with seroconversion, but not in plasma of 150 seronegative donors or of 598 donors who had been seropositive for at least 1 year, 148 of whom even had elevated ALT. Also ambiguous results of the TaqMan PCR were detected only in connection with seroconversion. Those results may represent CMV DNA concentrations below the 95 percent detection limit, but to ensure a conservative interpretation of the data, they had been excluded from analysis. The presence of active infection in newly seropositive donors with detection of CMV DNA in plasma is confirmed by a significantly higher percentage of donors with elevated levels of neopterin and ALT compared to seroconverted donors without detection of CMV DNA.

CMV DNA in plasma of seronegative donors during the "window period" of CMV infections was rare in our study, because CMV DNA was detected in the last seronegative sample of only 2 of 68 donors (3%). This is confirmed by Drew and colleagues[17] who studied the last seronegative sample of 192 seroconverting donors, finding only 1 DNA-positive sample (0.5%). In contrast, 2 donors in our study were CMV DNA–positive for at least 84 or 98 days, respectively. Further studies are necessary to determine the duration of CMV DNAemia in asymptomatic immunocompetent persons and to calculate the risk of window-period donations.

Even if the percentage of donors with abnormal surrogate markers like elevated ALT, elevated neopterin, or low WBC count was significantly higher in CMV DNA–positive donors compared to CMV DNA–negative donors, the sensitivity for detection of CMV DNA–positive donations with all these unspecific markers for infectious diseases was no more than 71 percent. Usually, cutoff limits for surrogate markers are set beyond the normal range to

prevent the exclusion of unnecessarily high numbers of donors.[37] This would further decrease the sensitivity of ALT and WBCs, especially, with for example only 11 percent of CMV DNA–positive donors having ALT values outside the former German limits for blood donation. This is in contrast to the results of Drew and coworkers[17] who reported both seroconverted donors with CMV DNA as being excluded from donation because of elevated ALT (92 and 117 U/mL, respectively). As reported by others[38] neopterin could be an option for blood donor screening with a sensitivity of 61 percent in our study. More effective prevention of CMV transmission could be achieved by transient exclusion of newly seroconverted donors. This was already suggested by Beneke and coworkers[39] who found a correlation between anti-CMV IgM-positive donors and TT-CMV. Lamberson and coworkers[40] confirmed that IgM-positive donors were responsible for TT-CMV in 7 of 70 seronegative neonates, whereas the only case of TT-CMV in 87 seronegative neonates after transfusion of anti-CMV IgG-positive and IgM-negative blood was explained by a false-negative result of the IgM assay.

The period of exclusion is difficult to determine on the basis of our data. The last CMV DNA–positive sample was drawn 84 days after the first seropositive donation, but additional samples were only available for 2 of 4 donors who tested CMV DNA–positive in their second seropositive sample. Thus, we cannot determine the possible duration of CMV DNAemia after seroconversion. In contrast, DNA-negative samples were available from 59 of 82 newly seropositive donors earlier than 1 year after the first seropositive sample. Furthermore, 598 donors (148 of whom even had elevated ALT), who had been seropositive for at least 1 year, tested negative for the presence of CMV DNA. This results in a 95 percent CI for the prevalence of CMV DNA–positive donors of less than 0.5 percent in this donor population. So exclusion of newly seroconverted donors for a period of 1 year after seroconversion seems to be sufficient to avoid TT-CMV.

A seasonal reactivation of latent CMV infections reported by Dumont and colleagues[16] could not be found in our donors. CMV reactivation in response to environmental allergens, which was suggested by Dumont and colleagues, could account for the differences, because a correlation between reactivation and pine tree pollen season was reported and those pollen are not a relevant allergen in Germany. A simpler explanation would be the use of systemic steroids in hay fever therapy,[41] which is very rare in Germany, but no data are available on this.

Given that 1 of every 1,000 or 10,000 peripheral blood monocytes from healthy CMV-positive individuals is supposed to be latently infected with a range of 2 to 13 geq per cell,[42,43] the number of latently infected monocytes in WBC-depleted red blood cell (RBC) units was estimated to be up to 50 and the CMV DNA concentration equals approximately 10^2 to 10^3 geq per unit.[44] Our study yielded comparable results for the mean CMV DNA concentration in connection with seroconversion ranging from 10^3 geq per unit in plasma-reduced RBC units to 10^4 geq per unit in fresh-frozen plasma or platelet (PLT) concentrates.

The seroconversion rate of 0.8 percent in our donor population corresponds well to the results of others, who found a seroconversion rate among healthy blood donors of between 0.2 and 1.2 percent.[38,45,46] In a recent meta-analysis Vamvakas[13] reports the risk of TT-CMV after transfusion of WBC-reduced components as being 2.73 percent versus 1.45 percent after transfusion of components from seronegative donors. He concludes that CMV-seronegative blood components are more efficacious in preventing TT-CMV than WBC-reduced components. Reviewing the included studies as well as other studies about TT-CMV after transfusion of WBC-reduced blood components shows great variations both in the rate of TT-CMV and in the amount of blood products transfused (Table 5).

Under the assumption that the prevalence of CMV DNA in blood products due to primary CMV infections was equal in the studies' donor populations to the minimum prevalence calculated for our institution (0.13%), the percentage of patients who had been transfused with CMV DNA–positive blood can be calculated with the previously explained equation:

$$\%\text{Patients} = 100 \times [1 - (1 - 0.0013)^{\text{number of units transfused}}].$$

With the exception of the study of Ohto and colleagues,[47] this percentage is about equal to or even higher than the percentage of patients actually developing TT-CMV. There is no correlation between the rate of TT-CMV and the proportion of patients potentially transfused with CMV DNA–positive blood. This may be due to differences between patient populations as well as donor populations, because no study provides information about the rate of CMV DNA–positive blood components or about factors influencing it such as, for example, the proportion of newly seroconverted donors relative to all seropositive donors or the length of interdonation intervals of first-time seropositive donors.

The study of Ohto and coworkers[47] differs from the other studies, because 94 percent of the studied neonates were fed with milk from their seropositive mothers. Therefore, the authors conclude that the observed CMV infections are probably unrelated to transfusions.[47]

In the retrospective study of Nichols and associates,[12] only 6 percent of the transfused units had been WBC reduced of CMV-seropositive donors, whereas 94 percent were unfiltered units of seronegative donors. A multivariate analysis identified only filtered RBCs from seropositive donors associated with an elevated risk for TT-CMV of a 32 percent relative or about 1 percent (0.2%-2%) absolute

TABLE 5. CMV infection after transfusion of WBC-depleted blood products and patients potentially transfused with CMV DNA–positive blood due to primary CMV infections*

Report (year)	Sample size	Mean number of transfused units	Percent with CMV infection	Percentage of patients potentially transfused with CMV DNA–positive blood†
Murphy et al. (1988)[48]	11	43	0	5.6
Bowden et al. (1989)[49]	17	153	0	18.4
De Graan-Hantzen et al. (1989)[50]	59	69	0	8.8
Gilbert et al. (1989)[8]	30	2	0	0.3
De Witte et al. (1990)[51]	28	52	0	6.7
Bowden et al. (1991)[52]	35	189‡	0	22.2
Eisenfeld et al. (1992)[53]	48	9	0	1.2
Van Prooijen et al. (1994)[54]	60	65	0	8.3
Bowden et al. (1995)[9]	250	102	1.2-2.4§	12.7
Narvios et al. (1998)[14]	45	141‖	2.2	17.1
Ohto et al. (1999)[47]	33	3	9.1¶	0.4
Pamphilon et al. (1999)[55]	62	Not specified	0	Not applicable
Nichols et al. (2003)[12]	807	24**	3.0	3.1
Narvios et al. (2005)[15]	72	55-77††	2.8	7.1-9.7

* This table summarizes studies included in the meta-analysis of Vamvakas[13] as well as two additional studies.[14,52]
† Estimated according to the mean number of units blood transfused in the respective study and to an assumed prevalence of CMV DNA in the whole donor population due to primary CMV infections of 0.13 percent, with the previously explained formula: %patients = $100 \times [1 - (1 - 0.0013)^{\text{number of units}}]$.
‡ 164 units of WBC-reduced PLTs and 25 unfiltered RBC units from seronegative donors.
§ Dependent on whether infections occurring between Day 0 and Day 20 are counted.
‖ Approximately 30 percent from unscreened donors and 70 percent from seronegative donors.
¶ 94 percent of neonates were fed with milk from CMV–seropositive mothers.
** 0.3 WBC reduced units from CMV-seropositive donors and 23.7 unfiltered units from seronegative donors.
†† Dependent on the number of donations pooled for random PLT concentrates.

increase per RBC unit. This is comparable with the rate of units from newly seropositive donors containing CMV DNA estimated for our donor population (0.28%). Because free CMV cannot be removed efficiently by WBC reduction, transmission of cell-free virus from newly seroconverted donors could be an explanation for at least some of the cases of TT-CMV after transfusion of WBC-reduced components.

We did not analyze whether residual WBCs in WBC-depleted blood components of newly seroconverted or latently infected donors contained CMV DNA. Nevertheless, it cannot be ruled out that the residual risk of TT-CMV with WBC-depleted blood components is mainly due to viremia in connection with seroconversion of blood donors. In this instance, transfusion of WBC-reduced blood components from seronegative donors would imply a greater risk of TT-CMV than transfusion of WBC-reduced blood from donors who have been seropositive for at least 1 year, because window-phase donations but no reactivation could be detected in our study. Therefore, there is an urgent need for further studies comparing the risk of TT-CMV after transfusion of WBC-reduced blood from seronegative donors and donors who have been seropositive for at least 1 year.

In conclusion, the detection of CMV DNA was closely related to the first detection of CMV IgG antibodies in up to 62 percent of our newly seroconverted donors, depending on the interval to the last seronegative donation. Otherwise, the probability of detection of CMV DNA in plasma of blood donors at least 1 year after seroconversion was lower than 0.5 percent. Window-phase donations occurred in only 3 percent of seroconversion cases. On the whole, the main source of blood products containing free CMV DNA were newly seroconverted donors. Thus, it is necessary to discuss whether those donors should be excluded transiently from blood donations. Furthermore, transfusion of WBC-reduced blood components from seronegative donors could imply a greater risk of TT-CMV than transfusion of WBC-reduced blood from donors who have been seropositive for at least 1 year, because window-phase donations but no reactivation could be detected in our study.

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別紙様式第2

別紙(3)-3

医薬品　研究報告　調査報告書

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一般的名称			研究報告の公表状況	West nile virus and blood product safety in Germany Journal of Medical Virology March 1, 2008, 80/3 (557-563)	公表国 米国	
販売名（企業名）	タココンプ（CSL ベーリング株式会社）					

研究報告の概要 137

問題点（WNV の献血への混入）

本研究プロジェクトは、ドイツの Robert-Koch 研究所、Bernhard-Nocht 研究所と Paul-Ehrlich 研究所の共同で実施され、WNV がドイツにおいて国民の健康と血液供給にとって脅威となりうるかを調査した。

9976 名のドイツの健康なドナー、78 名のドイツの薬物使用者及び 198 名の米国の抗 WNV 抗体陽性患者から採取された検体を用いて WNV-RNA を NAT で測定した結果、全て陰性であった。

欧州で採取された血漿分画製剤用の原料血漿 96 件及び東アジアで採取された血漿分画製剤用の原料血漿 51 件は全て陰性であったが、米国で採取された原料血漿は 174 件中 32 件が陽性であった。

さらに本研究プロジェクトは、パスツリゼーションの WNV の不活化を実験室レベルで評価した。人血清アルブミンに安定剤を加え、WNV をスパイクした各々の溶液を 60℃で 1-10 時間加熱し、ウイルス不活化を $TCID_{50}$ で評価したところ、同実験系で測定した SFV と BVDV に似た不活化過程が確認された。

血漿分画製剤の不活化で良く用いられるパスツリゼーション処理、S/D 処理、低 pH 処理は、原料血漿のエンベロープで覆われたウイルスを不活化するのに十分であることが過去の研究で証明されている。

血漿分画製剤の製造工程にウイルス不活化工程が含まれていれば、血漿プール中に WNV が混入していても、感染リスクは無いと結論している。

使用上の注意記載状況・その他参考事項等

報告企業の意見	今後の対応
本剤はドイツや米国の原料血漿を使用している。万一、原料血漿に WNV が混入していても、本論文のとおり本剤のパスツリゼーション処理により不活化されると考えられる。	今後とも情報収集に努める所存である。

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Journal of Medical Virology 80:557–563 (2008)

West Nile Virus and Blood Product Safety in Germany

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West Nile Virus (WNV) is a mosquito-transmitted flavivirus, widely distributed throughout Africa, Asia and the Middle East. WNV may cause epidemics of human meningoencephalitis. The unexpected emergence of WNV (New York, 1999) and its rapid spread throughout North America during the following years caused a number of blood transfusion- and organ transplant-associated transmissions of WNV. In order to estimate the potential WNV threat for Central Europe, we analyzed the anti-WNV prevalence and WNV-RNA incidence among 14,437 and 9,976 blood donors from Germany. There was a high rate of initially anti-WNV reactives (5.9%), but only a few cases (0.03%) were confirmed as anti-WNV positive by neutralization assay. No WNV-RNA positive blood donor was identified in this study. Whereas WNV-RNA was frequently detected in manufacturing plasma pools from the US, none was detected in pools of European or Asian origin. Virus inactivation steps integrated into the manufacturing process of plasma derivatives were shown to be sufficient to assure the WNV safety of plasma derivatives. A well-characterized WNV reference material was prepared, showing 340 WNV-RNA copies per infectious dose. ***J. Med. Virol. 80:557–563, 2008.***

KEY WORDS: West Nile Virus; WNV neutralization; anti-WNV IgG ELISAs; blood safety; plasma derivatives; virus inactivation

INTRODUCTION

West Nile Virus (WNV) was identified in 1937 in Uganda and is widely distributed throughout Africa, Asia, the Middle East and parts of Europe [Solomon et al., 2003]. This enveloped virus is classified under the virus family *Flaviviridae* in the genus *Flavivirus*, which includes more than 100 members that all are characterised by a complex replication cycle involving both insects and at least one additional animal species. This is why flaviviruses had been included in the previous taxonomic group of arboviruses (arthropod-borne viruses). The natural WNV replication cycle involves *culicine* mosquitoes and different bird species. Humans, horses and other mammalian species are so-called "dead-end" hosts characterised by WNV infections with potential clinical symptoms, but transient and low virus levels that are insufficient to establish a mosquito-mammalian WNV replication cycle. The vast majority of WNV infections in humans undergo an asymptomatic course. Approximately 20% of infected humans develop West Nile fever, a febrile illness of sudden onset, often associated with a long recovery period. Only a few cases (<0.2%) develop a neuroinvasive disease resulting in more serious symptoms, including meningitis or encephalitis, sometimes with fatal outcome [Petersen and Marfin, 2002]. The rate of serious outcome of WNV infection is much higher in immune-compromised patients, a status more frequently found in elderly persons or in recipients of blood transfusions.

After a flavivirus infection, the diagnostic differentiation of specific antibodies is complicated by a high rate of cross-reactivity between different members of the genus Flavivirus, for example, Dengue virus (DenV), tick-borne encephalitis virus (TBEV), and WNV [Allwinn et al., 2002; Koraka et al., 2002]. Cross-reactive antibodies are mainly directed against an envelope protein of flaviviruses, the E-protein.

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The unexpected emergence of WNV in New York in 1999 was associated with an encephalitis outbreak. The viral strain responsible for this epidemic was a lineage 1 virus already known from previous epidemics in other parts of the world, such as in Israel [Lanciotti et al., 1999]. In the following years, WNV spread across North America from East to West, causing the largest arbovirus epidemic in recorded history with more than 23,000 human infection cases until December 2006, including 893 deaths [Centers for Disease Control and Prevention, 2006]. During this WNV epidemic, new transmission routes, including breast-feeding [MMWR, 2002], organ transplantation [Iwamoto et al., 2003] and transfusion of blood components [Biggerstaff and Petersen, 2002; Hollinger and Kleinman, 2003], were recorded. To assure the safety of the US blood supply, the screening of all blood donations by nucleic acid amplification techniques (NATs) was recommended by the FDA beginning in July 2003. This measure resulted in the detection of WNV-RNA in more than 1,000 blood donations until the middle of 2005, which would otherwise have been used for transfusion of non-inactivated cellular blood components (red cells, platelets) or therapeutic plasma [Busch et al., 2005a; Stramer et al., 2005].

Despite some reports about sporadic WNV infections in humans and horses across Europe, for example, in France, Italy, and Romania [Zeller et al., 2004], no WNV epidemiology data were available for Germany. With the US epidemic still ongoing, the question arose as to whether a similar scenario could also affect Europe. The German Ministry of Health initiated an investigation into the prevalence and incidence of WNV infections in Germany. This publication relates to the assessment of WNV safety of blood and plasma products used in Germany.

MATERIALS AND METHODS

Human Serum and Plasma Samples

For the evaluation of WNV prevalence, 14,437 plasma or serum samples were collected during Summer 2004 from healthy blood donors from central Germany (Hesse) and 928 samples from Austrian blood donors (Carinthia and Vienna).

For the determination of WNV incidence, plasma samples from 9,978 healthy blood donors from central Germany (Hesse) were collected during the 2005 summer mosquito season, combined in pools of 8 using a Tecan robot, and tested for the presence of WNV-RNA.

Serum samples from German intravenous drug users (IVDUs) who tested positive for other blood-borne viruses (HIV and/or HCV) were collected during May 2002 and January 2004 at University Hospital Frankfurt/Main.

Anti-WNV IgG-positive plasma samples were obtained from confirmed clinical cases in the US. These plasma samples were obtained from BBI (West Bridgewater, MA) or kindly provided by H. Hofmann (Genzyme Virotech GmbH, Mainz, Germany).

One panel of WNV RNA-positive plasma samples from clinical cases in the US was provided by L. Tobler from the Blood Systems Research Institute, San Francisco.

Panels of anti-DenV and anti-TBEV positive specimens were designed with materials obtained from the respective clinical cases in Germany, which were kindly provided by Universität München, Abt. für Infektions und Tropenmedizin (T. Löscher), Universität Freiburg, Institut für Medizinische Mikrobiologie & Hygiene (D. Neumann-Haefelin), Universität Heidelberg, Hygiene Institut (P. Schnitzler) and Universität Frankfurt, Zentrum für Hygiene (R. Allwinn).

Aliquots of plasma pools collected for the fractionation of plasma derivatives were obtained from different plasma manufacturers. The respective plasma units had been collected in the US, in Europe or in Asia during the years 2004 and 2005.

ELISA Tests

The following ELISAs were performed strictly following the instructions given in the package insert: "Flavivirus IgG indirect ELISA" (PANBIO Brisbane, Australia), "Anti-West-Nile-Virus-ELISA IgG" (prototype version, Euroimmun, Lübeck, Germany), "West Nile Virus IgG DxSelect™" (Focus Diagnostics, Cypress, CA), and "Enzygnost anti-TBE Virus IgG and IgM" (DADE Behring, Marburg, Germany).

WNV NATs

Human plasma or serum and cell culture-derived samples were tested for WNV RNA using the "Procleix WNV assay" (Chiron Corporation, Blood Testing Division, Emeryville, CA). This test system is a qualitative nucleic acid amplification technique (NAT) based on TMA (transcription mediated amplification) technology. This NAT was performed after passing a training seminar organized by the manufacturer and strictly following the manufacturer's instructions.

WNV-RNA was quantified with the Artus Real Art™ WNV LC RT RCR Kit (QIAGEN, Hilden, Germany) using the QIAamp Viral RNA Mini Kit (QIAGEN) for viral RNA extraction. Quantification standards were provided with the test kit and run in parallel.

Viruses and Cells

Bovine viral diarrhea virus (BVDV), strain Osloss, was obtained from G. Pauli, Robert Koch Institut, Berlin, Germany, and was propagated and titered in MDBK cells (ATTC CCL-22). SFV was provided by J. Thiel (Institut für Virologie, Universität Giessen, Germany), and was propagated and titered in Vero cells (ATTC CCL-81). WNV (1999 New York isolate) was received from T.R. Kreil (Baxter, Vienna, Austria), and was propagated and titered in Vero cells in a biosafety level 3 laboratory.

Virus Titration

Virus infectivity was quantified by estimation of the tissue culture infectious dose ($TCID_{50}$) using standard cell culture conditions. Briefly, Vero and MDBK cells were grown to confluence in 96-well microtiter plates (MTPs). Threefold serial dilutions of the samples in

DMEM medium were prepared, and eight replicates per dilution were assayed by inoculation of a 50 µl sample per well. The cytopathic effects of WNV, Semlilki Forest Virus (SFV), and BVDV were checked on days 3–6 post-infection. The $TCID_{50}$ was calculated according to the Maximum Likelihood statistical tool. If no virus was detected, the limit of detection was calculated according to the Poisson distribution.

WNV Neutralization Assay

For the WNV neutralization assay, 50 µl of a 1:3 serial dilution (1:10 to 1:270) of inactivated (56°C, 30 min) serum was mixed in eight replicates in MTP wells with 50 µl of 20 $TCID_{50}$ units of virus. After incubation for 60 min at 37°C, 50 µl of each well was transferred to another well containing 60% confluent (6,000–7,000) Vero cells. MTPs were incubated for 5–6 days at 37°C, and wells containing evidence of viral cytopathic activity were scored. The log ND_{50} was calculated according to the Maximum Likelihood function [Kundi, 1999]. For a high throughput version of the neutralization assay, only one serum dilution (1:30) was tested under the same conditions described above.

Virus Inactivation Studies

Pasteurization was performed at laboratory scale to assess this virus inactivation step. Stabilizer (1.1 g/ml saccharose, 0.3 g/ml glycine, 0.0162 g/ml $CaCl_2$ dihydrate) was added to 50 ml of a commercial 5% human normal immunoglobulin preparation. The solution was spiked with virus and heated to 60°C. Samples were taken after time intervals as indicated (0–10 hr) and immediately cooled. Cooled samples were subsequently titered. Before application on indicator cells, samples were diluted 1:100 in order to avoid cytotoxic and interfering effects of the test material. Absence of cytotoxic effects was verified by microscopic examination of control cells inoculated with non-spiked 1:100 diluted test material. Absence of interfering effects was verified by positive detection of virus from diluted (1:100) test material that had been spiked with a known amount of virus (100 $TCID_{50}$ per well).

RESULTS

Sensitivity and Specificity of Anti-WNV IgG ELISAs

To assess the relative diagnostic sensitivity and specificity of three anti-WNV IgG ELISAs, we used sera from well-characterized flavivirus-infected patients: 26 WNV-infected, 39 TBEV-infected, and 13 DenV-infected individuals.

Two assays (PANBIO, Focus) recognized all of the 26 anti-WNV-positive sera originating from U.S. patients. The third ELISA, the Euroimmun prototype ELISA version, missed three specimens, resulting in a relative sensitivity of 88% in this study (Table I).

The cross-reactivity rates with related flaviviruses were determined for the anti-WNV ELISAs using specimens from DenV or TBEV-infected patients. The highest cross-reactivity rate (lowest specificity) with these specimens was obtained for the PANBIO assay (92.3% and 79.5%, respectively) followed by the Focus ELISA (92.3% and 56.4%, respectively) and the Euroimmun prototype ELISA, which displayed cross-reactivity rates of 35.7% and 17.9%, respectively.

Based on this analysis of the relative sensitivity and specificity of the different WNV ELISA assays, the following test algorithm for determination of the WNV prevalence was chosen: after screening donor blood with the sensitive Focus ELISA test, a further analysis of all reactive specimens was performed with the more specific Euroimmun prototype WNV ELISA. Those samples that were reactive (positive or borderline) in the second anti-WNV ELISA were then tested in a WNV neutralization assay as a confirmatory test. Additionally, we characterised all samples reactive in the Focus WNV ELISA with an anti-TBEV IgG ELISA. However, this assay displayed 42% cross-reactivity when anti-WNV-positive specimens were tested.

WNV Prevalence

More than 14,000 healthy blood donors from Hesse/Germany were screened for anti-WNV specific IgG antibodies. 5.9% (852/14,437) of the tested donors were reactive in the Focus anti-WNV ELISA test. To estimate the potential impact of TBEV vaccination on the test results, more than 900 blood donors from Austria (where TBEV vaccination is a general public health measure) were also screened for anti-WNV. Seventy-two percent of these blood donors (669/928) were reactive in the anti-WNV ELISA.

All anti-WNV reactive samples from German blood donors were re-tested in the second anti-WNV ELISA and in the anti-TBEV IgG assay. Thirty-four percent of these samples were reactive in the second anti-WNV and 15% were non-reactive in the anti-TBEV ELISA, whereas 9.7% were reactive in the WNV-ELISA and non-reactive in the TBEV-ELISA.

TABLE I. Sensitivity and Cross-Reactivity of Different Anti-WNV IgG ELISAs (pos = Positive; React = Reactive)

		Anti-WNV IgG ELSA		
	Sera	PANBIO	Focus	Euroimmun
Sensitivity	Anti-WNV IgG positive	100% (26/26)	100% (26/26)	88% (23/26)
Cross-reactivity	Anti-dengue IgG positive	92.3% (12/13)	92.3% (12/13)	35.7% (5/14)
	Anti-TBE IgG positive	79.5% (31/39)	56.4% (22/39)	17.9% (7/39)

Two hundred two of the pre-selected anti-WNV suspicious blood donor samples were tested in the WNV neutralization assay. Most of these plasma samples (148/202; 73%) had no or very low WNV neutralization activity (log $ND_{50} < 1$), while 50 specimens (25%) neutralized WNV infection, with log ND_{50} titers between 1 and 2. Only four plasma samples from our blood donors exhibited relatively high neutralization titers (log $ND_{50} \geq 2$) equivalent to those titers observed with the anti-WNV IgG sera from US patients. To check whether any anti-WNV positive might have been missed by our test algorithm, 388 additional anti-WNV reactive specimens were tested in a high throughput neutralization assay. No further specimens with titers of log $ND_{50} > 2$ were identified by this approach.

WNV Incidence

The WNV incidence in a population of healthy German blood donors was investigated during the mosquito season of Summer 2005. In total, 9,976 blood donors were tested for the presence of detectable WNV-RNA using the Procleix WNV NAT assay in minipools of eight. All of the 1,247 minipools tested WNV-RNA negative, with the exception of one initially reactive test result for one minipool. This result was not confirmed on retesting and on testing of the individual plasmas. Furthermore, 198 of the anti-WNV reactive blood donor samples (see above) were tested as individual specimens, with negative test results. Plasma or serum samples collected from a population of German intravenous drug users (IVDU, n = 78), representing a population with increased risk for blood-borne pathogens also tested negative for WNV-RNA (Table II).

Plasma Derivatives

Plasma pools for manufacturing of plasma derivatives with the source plasma collected in the US, East Asia, or Europe were analyzed for the presence of WNV RNA using the Chiron TMA assay. All plasma pools from East Asia (n = 51) and Europe (n = 96) tested negative, while 32 out of 174 (18%) plasma pools from the US tested WNV RNA-positive in this qualitative WNV-NAT (Table II). These pools had been collected in different regions of the US during the years 2004 and 2005. Since the viral load in most pools was too low for accurate results in the quantitative WNV-NAT, viral load was analyzed after concentration of WNV particles by ultracentrifugation and subsequent extraction of the viral nucleic acids. Viral loads were calculated as ranging from 57 to 837 copies WNV-RNA/ml plasma, with 351 copies WNV-RNA/ml as an average value for the TMA-positive pools.

TABLE II. Detection of WNV RNA in Blood Specimens and Plasma Pools Using the Procleix WNV Assay (Chiron)

	Tested	WNV-RNA positive
Blood donors (pools of n = 8)	9,976	0
IVDUs	78	0
Anti-WNV reactive blood donors	198	0
Plasma pools (Europe)	96	0
Plasma pools (USA)	174	32
Plasma pools (East-Asia)	51	0

Virus inactivation steps are included in the manufacturing process of different plasma derivatives. For the production of human-derived medicinal products, a 10 hr heating step of a liquid product intermediate at 60°C (pasteurization) is often performed to inactivate a wide range of potential virus contaminants. The inactivation kinetics of WNV upon pasteurization of a sucrose-stabilized immunoglobulin preparation was compared with the inactivation kinetics of other commonly used model enveloped viruses. BVDV is a pestivirus that frequently serves as a model virus for hepatitis C virus and other members of the Flaviviridae. The inactivation kinetics of WNV were similar to the inactivation kinetics of BVDV. The inactivation kinetics (Fig. 1) confirm that WNV is effectively inactivated by this commonly used manufacturing step.

WNV Reference Preparation

We established and characterized a WNV reference preparation that may be useful for standardization and control of WNV-NATs and WNV-neutralization assays. Supernatant from WNV-infected Vero cells was harvested and characterized for its infectivity titer ($TCID_{50}$) and WNV-RNA content. WNV-RNA concentration was determined by replicate (24 per concentration) testing of limiting dilutions (factor of 2) using the qualitative WNV-NAT followed by calculation of the 95% cut-off concentration using Probit analysis. WNV-RNA concentration was also determined using the quantitative NAT test.

Both NAT approaches revealed a WNV-RNA concentration for the stock material of 6.5×10^9 or 8.1×10^9 copies/ml. Titration in Vero cells gave an infectivity titer of log 7.33 TCID/ml, correlating to approximately 340 copies WNV-RNA per infectious dose.

DISCUSSION

New emerging pathogens may be a threat to public health, not only because of their impact on the population, but also because of their potential to contaminate the blood or plasma supply and to be transmitted to recipients of blood products. Therefore, a research project was initiated by the German Ministry of Health after the huge WNV epidemic that followed the introduction of the virus to the New World in 1999. This research project was performed in cooperation among the Robert-Koch-Institut (RKI; Berlin), the Bernhard-Nocht-Institut (BNI; Hamburg), and the Paul-Ehrlich-Institut (PEI; Langen). In this study, we investigated whether WNV is or could become a threat to public health and the blood supply in Germany. Here, we focus on the prevalence and incidence of WNV among healthy blood donors and the potential for the transmission of the pathogen via plasma derivatives.

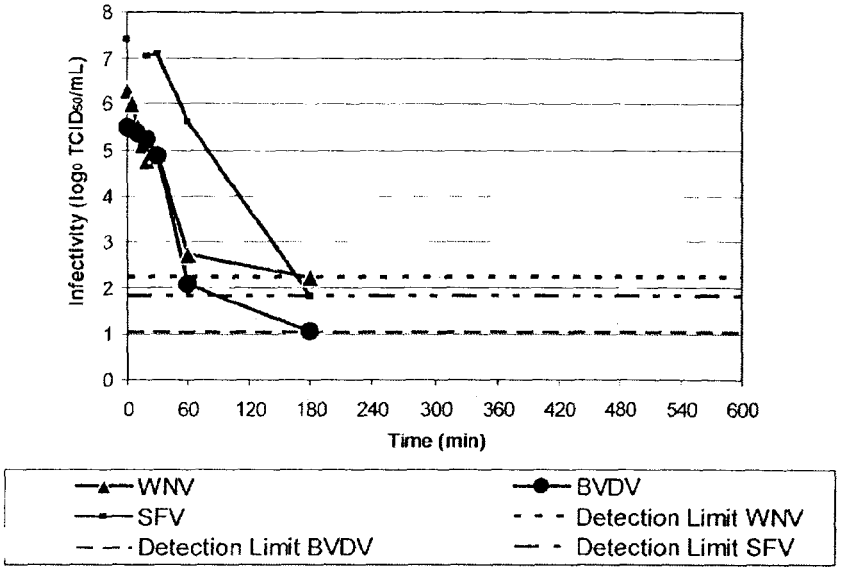

Fig. 1. Virus inactivation through heat inactivation (pasteurization). A sucrose-stabilized human immunoglobulin preparation (5%) was spiked with BVDV, SFV or WNV. After incubation at 60°C, samples were taken after time intervals as indicated (0–10 hr) and cooled immediately. Virus infectivity was quantified by calculation of the tissue culture infectious dose ($TCID_{50}$).

WNV Prevalence

First, we wanted to qualify the anti-WNV screening tests and define an appropriate test algorithm. We decided to use anti-WNV positive specimens from the US as decisive specimens for testing sensitivity for different reasons. First, the respective materials from clinically ill patients were easily available from the US. Second, WNV infections had been confirmed by the clinical course, the seasonal occurrence of the infection and the results of different diagnostic assays performed on individual follow-ups. Furthermore, TBEV, the human flavivirus most frequently found in Europe, is not yet present on the American continent. Therefore, cross-reacting TBEV antibodies should not be an issue for these samples.

The sensitivity was 100% for two anti-WNV assays and 88% for the third assay using such an anti-WNV positive serum panel. However, further panels composed of either anti-Dengue or anti-TBEV positive specimens showed high rates of cross-reactivity with the more sensitive anti-WNV assays and low cross-reactivity with the third assay. We decided to include an anti-TBEV assay as a further diagnostic tool because all of the anti-WNV positive specimens from the US that were missed by the third assay tested as anti-TBE negative, and only a few members of the entire US anti-WNV panel cross-reacted in the anti-TBEV assay. We aimed to preferentially choose anti-WNV reactive/anti-TBEV negative specimens for entering the "gold standard" WNV neutralization test. However, even with this standard, we experienced a high rate of cross-reactivity with anti-DenV-positive specimens. Fortunately, anti-TBEV-positive specimens, which are much more frequent in our region, showed cross-neutralization only at a low level. In conclusion, serological flavivirus diagnostics show high rates of cross-reactivity, and correct interpretation of test results requires extreme caution.

To determine the extent of past WNV infections, samples from more than 14,000 German blood donors were screened with the Focus anti-WNV IgG ELISA. Nearly 6% of the German blood donors were anti-WNV reactive in this assay. Many of the reactive test results were probably caused by cross-reactive antibodies originating from a related flavivirus infection or from vaccination. Though some parts of Southern Germany are TBE risk areas (as the clinical cases show), no reliable TBE incidence or prevalence data are available for Germany.

The TBE vaccination coverage of the Austrian population is in the range of 80%. Sera from Austrian blood donors had a similar reactivity rate in our anti-WNV screening assay. This illustrates the high rate of anti-TBEV cross-reactivity and confirms the similar results obtained in our test qualification study (see above).

Therefore, the anti-WNV reactive rate for blood donors in Hesse (5.9%) may primarily reflect the TBEV vaccination and/or infection level in donors from our region.

To narrow down the number of samples that had to be tested in a WNV neutralization assay, all blood donations that were reactive in the Focus ELISA were re-tested in the more specific prototype anti-WNV IgG ELISA test and in an anti-TBEV IgG assay.

Blood samples that were non-reactive in the anti-TBEV ELISA and reactive in the pre-market anti-WNV

ELISA were tested in the WNV neutralization assay, the "gold standard" for antibody detection, to confirm a past infection. Most of the pre-selected specimens did not inhibit WNV infection in cell culture at all or did so only at a low level (log $TCID_{50} < 1.5$). The titer of 13 additional samples was between ≥ 1.5 and <2.

Only four blood donor samples exhibited a log titer of >2, which was similar to those titers we determined for different clinically confirmed WNV positive sera from the US (Table I). We interviewed the four anti-WNV positive blood donors to evaluate if these putative WNV infections occurred in Germany or Europe or if they might be associated with WNV-endemic countries. One of the four donors had never left Europe, but had visited South and East Europe; another donor had only stayed outside Germany in Turkey. The other two donors had traveled abroad to the USA and Africa. We therefore cannot exclude that singular sporadic and asymptomatic WNV infections may have occurred in Europe and the Middle East. However, WNV prevalence among German blood donors is overall very low; in our study, only 0.03% (95% CI: 0.01–0.07%) of blood donors that were tested were confirmed positive for anti-WNV antibodies by our approach.

WNV-RNA Incidence

For WNV infections, a diagnostic window of only few days (until detection of anti-WNV) has been described during which viral RNA may be detectable. With a WNV-RNA concentration range described from <50 copies/ml up to 10^5 copies/ml [Busch et al., 2005b], the short viremia is at a moderate level when compared to the respective figures for other blood-borne viral infections like HCV (3 months, 10^5–10^8 IU/ml) or HIV (4 weeks, 10^2–10^6 IU/ml) [Lelie et al., 2002].

WNV-RNA incidence in Germany was analyzed in minipools comprised of eight blood donations collected during Summer 2005 and using the highly sensitive Chiron Procleix WNV NAT [Gallian et al., 2005]. Since different WNV strains were described as circulating in Central Europe [Bakonyi et al., 2005, 2006], we investigated whether the Procleix assay detects all available WNV strains. A panel from an external quality assurance study [Niedrig et al., 2006] was retrospectively analyzed. The result confirmed the capability of the Procleix assay to detect WNV lineage 1 and 2 with high sensitivity (data not shown).

No minipools were confirmed positive for WNV-RNA after screening of 1,247 minipools (equal to 9,976 donations). Negative NAT results were also obtained for all individually tested samples from a population that was at increased risk for blood-transmitted infections [i.e., intravenous drug users (IVDUs, n = 78)], or for plasma samples of serologically anti-WNV-reactive blood donors (n = 198).

This outcome is in accordance with the recent WNV-RNA screening results of more than 60,000 Dutch blood donors [Koppelman et al., 2006]. Regardless, blood donors who had just recently visited a WNV endemic country (e.g., the USA) are excluded from the actual donation as a precautionary measure in German blood banks.

Plasma Derivatives

Plasma derivatives (e.g., immunoglobulin preparations, albumin, factor concentrates) may enter the European market with source materials collected in different parts of the world. We were interested in the question of whether WNV-RNA is detectable in aliquots of manufacturing plasma pools.

Some of the plasma pools with plasma of US origin tested positive for WNV RNA by the qualitative NAT assay. The viral load in these plasma pools was determined in the range between 57 and 837 copies/ml, with a median of 351 copies/ml. Each of these pools was composed of several thousand plasma units. Assuming that WNV input in these pools originates from only few donors, WNV concentration of 10^5–10^6 copies/ml are calculated for some individual plasma units. Such high WNV-RNA concentrations have been described for the diagnostic window phase in few cases. However, WNV input into plasma pools from lower viremia cases may be not detected due to the dilution effect though they are expected to be much more frequent and representative for the early infection phase.

Several studies proved that inactivation steps commonly used during the manufacture of plasma derivatives, such as pasteurization for human albumin, S/D treatment for IVIG and FVIII inhibitor-bypassing activity, and incubation at low pH for IVIG, should be sufficient to inactivate enveloped viruses present in source materials [Kreil et al., 2003]. We comparatively investigated the efficacy of pasteurization with regards to WNV and model viruses for enveloped viruses. In these experiments WNV inactivation kinetics were similar between WNV and the two model viruses, BVDV and SFV. This result confirmed the validity of predictions based on model viruses if chosen appropriately. It allowed the conclusion that moderate WNV concentrations in plasma pools should not pose an infection risk to recipients if virus inactivation procedures are included in the manufacturing process of these biological medicinal products.

CONCLUSION

There is currently no indication that WNV could cause an epidemic in Europe similar to that in the USA during the recent years, although temporally and regionally limited outbreaks of WNV infections in humans and horses have been observed in Europe since the 1950s. In contrast to North America, Europe has had direct vector contact with WNV endemic areas in Africa for a long time, via migratory birds, for example. This may have resulted in natural herd immunity in birds. In America, a highly pathogenic WNV strain was imported in 1999 into a virgin territory, meeting a bird population without herd immunity. Environmental factors, such as climate change or global warming and the increasing

mobility of people, may enhance the emergence of new viruses. Therefore, continuous surveillance is an important tool to protect public health and the safety of the blood supply.

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No. 27

医薬品 研究報告 調査報告書

識別番号・報告回数			報告日	第一報入手日	新医薬品等の区分	機構処理欄
				2007. 12. 4	該当なし	

一般的名称	(製造承認書に記載なし)	研究報告の公表状況	ProMED 20071130-3869, 2007 Nov 30. 情報源:[1]World Health Organisaation (WHO), CSR, Disease Outbreak News, 2007 Nov 30. [2]Agence France Press (AFP) report, 2007 Nov 30.	公表国
販売名(企業名)	合成血「日赤」(日本赤十字社) 照射合成血「日赤」(日本赤十字社) 合成血-LR「日赤」(日本赤十字社) 照射合成血-LR「日赤」(日本赤十字社)			ウガンダ

研究報告の概要

○ウガンダにおけるエボラ出血熱アウトブレイク

[1]ウガンダの保健省は西部のBundibugyo地区でのエボラ出血熱アウトブレイクを確認した。2007年11月28日時点で疑い症例51例、うち死亡例16例が報告されている。報告例には医療関係者の感染も3例含まれており、このうち1例は死亡している。国立研究所および米国疾病対策予防センター(CDC)の実施した臨床検査により、患者検体からエボラウイルスの新種の存在が確認された。現地調査によると、アウトブレイクは2007年9月から始まっていた可能性がある。同国保健省の対策委員会、WHOや他の国際機関は協力して対応に当たっていく。

[2]2007年11月30日、ウガンダ保健省は、同国西部で51人が感染し、少なくとも16人が死亡したエボラウイルスは未知のウイルス株であると発表した。CDCの検査施設に送られた患者の血液及び組織検体を分析したところ、これまでウガンダの他の地区やコンゴ民主共和国で流行していたエボラウイルスの株とは異なった性質が見られた。専門家によると、これまでの株は血管の内膜を破壊することで出血を引き起こし、ショックによって患者を死に至らしめるが、新しい株では出血はそれほど多くなく、患者は高熱を発症後に死亡するとのことである。当局は疫学やウイルス学の専門家を集めて同地区の疾患を監視し、高熱や腹痛、嘔吐、紅斑を発症した人に注意している。

報告企業の意見	今後の対応
ウガンダ西部のBundibugyo地区でエボラ出血熱の集団発生が見られ、エボラウイルスの新種の存在が確認されたとの報告である。	日本赤十字社は、輸血感染症対策として問診時に海外渡航歴の有無を確認し、帰国(入国)後4週間は献血不適としている。今後も引き続き情報の収集に努める。

使用上の注意記載状況・その他参考事項等

合成血「日赤」
照射合成血「日赤」
合成血-LR「日赤」
照射合成血-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

Back

Archive Number 20071130.3869
Published Date 30-NOV-2007
Subject PRO/AH/EDR> Ebola hemorrhagic fever - Uganda: (Bundibugyo), WHO

EBOLA HEMORRHAGIC FEVER - UGANDA: (BUNDIBUGYO), WHO
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A ProMED-mail post
<http://www.promedmail.org>
ProMED-mail is a program of the
International Society for Infectious Diseases
<http://www.isid.org>

[1]
Date: Fri 30 Nov 2007
Source: World Health Organisaation (WHO), CSR, Disease Outbreak News [edited]
<http://www.who.int/csr/don/2007_11_30a/en/index.html>

Ebola haemorrhagic fever in Uganda

The Ministry of Health (MoH), Uganda, has confirmed an outbreak of Ebola haemorrhagic fever, in Bundibugyo District, western Uganda. As of Thu 28 Nov 2007, 51 suspected cases, including 16 deaths have been reported. Among the reported cases, 3 health care workers were also infected, including one fatality. The patients are being hospitalized at Kikyo and Bundibugyo.

Laboratory analysis undertaken at the National Reference Laboratories and the Centres for Disease Control and Prevention (CDC), Atlanta, USA, has confirmed the presence of a new species of Ebola virus in samples taken from cases associated with the outbreak.

Based on initial field investigations, the MoH/WHO Country office has reported that the outbreak might have been ongoing since September 2007. A national task force comprising MoH, WHO and other international partners in the field, is coordinating the response to this outbreak. WHO Country office is assisting the MOH national field team and the District health officials.

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Communicated by:
ProMED-mail Rapporteur Marianne Hopp

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[2]
Date: Fri 30 Nov 2007
Source: Agence France Press (AFP) report [edited]
<http://afp.google.com/article/ALeqM5j8JhykvCqpuWN9lWD4ZjGnYZeA_g>

Uganda's Ebola outbreak is new strain

A lethal Ebola virus that has killed at least 16 people and infected 51 others in western Uganda is a previously unknown strain, health authorities said Friday [30 Nov 2007]. Analysis on victims' blood and tissue samples sent to the Atlanta-based Centers for Disease Control's pathogens laboratory behaved differently from previous known strains of Ebola, they said. "It is a new type of strain. It is different from the one we suffered in Gulu and also different from the one that was reported in the Democratic Republic of Congo," said Sam Okware, who chairs Uganda's national hemorrhagic fever task force. The 1st Ebola case was reported on 10 Nov 2007 in Bundibugyo district on the border with the Democratic Republic of Congo, where 3

patients are currently in an isolation ward.

Virologists say previous strains destroyed the linings of blood capillaries and vessels, prompting fluids to drain out of the circulatory system through the body's orifices and pores, killing the victim through shock. But there is not much bleeding in the new strain that appears to kill its victims after provoking a high fever, they say.

Authorities have assembled epidemiologists and virologists in the affected district to monitor the disease. "We have put our people on alert for anyone who is complaining of fever, abdominal pain, vomiting and has developed rashes," Okware said, referring to the early symptoms of the disease caused by the new strain.

An outbreak of Ebola, a highly contagious disease that can have fatality rates as high as 90 percent, killed at least 170 people in northern Uganda's Gulu district in 2000. A similar outbreak has killed at least 26 people in the West Kasai region of the Democratic Republic of Congo in recent weeks, according to the country's Health Minister Victor Makwenge Kaput.

It spreads by direct human contact, especially through infected blood. The Ebola virus was first identified in 1976 in Sudan and in a nearby region of Democratic Republic of the Congo (then Zaire). Outbreaks of Ebola have also occurred in the Ivory Coast and Gabon.

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[The difference in symptoms of the disease associated with this new strain of Ebola virus, and its less hemorrhagic presentation, may account in part for the delay in identifying the causes of the outbreak. On the basis of the WHO report it must be presumed that the disease was present in the region for several weeks prior to recognition of the 1st case on 10 Nov 2007.

An interactive map of the Bundibugyo region of Western Uganda is available at <http://www.maplandia.com/uganda/bundibugyo/>. - Mod.CP]

[see also:
Hemorrhagic fever - Uganda (04): (Bundibugyo), Ebola confirmed 20071130.3859
Hemorrhagic fever - Uganda (03): (Bundibugyo) 20071121.3775
Hemorrhagic fever - Uganda (02): (Bundibugyo), Marburg NOT 20071116.3718
Hemorrhagic fever - Uganda (Bundibugyo): Marburg susp., RFI 20071114.3697
Marburg hemorrhagic fever - Uganda (06): new case 20071002.3257
Marburg hemorrhagic fever - Uganda (05) 20070817.2697
Marburg hemorrhagic fever - Uganda (04), WHO 20070814.2656
Marburg hemorrhagic fever - Uganda (03) 20070810.2609
Marburg hemorrhagic fever - Uganda (02) 20070804.2533
Marburg hemorrhagic fever - Uganda 20070803.2514
Viral hemorrhagic fever - Uganda (Kamwenge): Marburg conf. 20070801.2490
Viral hemorrhagic fever - Uganda (Kamwenge): Marburg susp., RFI 20070801.247
2001

Ebola hemorrhagic fever - Uganda: postscript 20011019.2576]
.....cp/ejp/lm

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研究報告の概要

○イタリアでのチクングニヤウイルス感染症:温帯地域におけるアウトブレイク

背景:ヤブカ類(Aedes spp.)によって伝播されるチクングニヤウイルス(CHIKV)は、近年インド洋諸島やインド亜大陸において複数のアウトブレイクを引き起こした。ここではイタリアでのアウトブレイクを報告する。

方法:イタリア北東部の隣りあった2つの村で原因不明の発熱性疾患が多数報告された後、主要感染源と伝播形態を特定するためのアウトブレイク調査を実施した。能動的サーベイランスシステムも導入した。臨床症例定義は、発熱と関節痛の発症とした。血液検体を採取、PCRと血清学的検査を行って病原体を特定した。現地で採取した蚊にもPCRを実施した。CHIKV E1領域の系統発生学的解析を行った。

知見:ヒトおよび蚊由来検体の分析により、当該アウトブレイクはCHIKVによるものと判明した。2007年7月4日から9月27日までにCHIKV感染症例205例が特定された。推定初発症例は、当該の村に親類を訪ねた際に発症したインドの男性とされた。系統発生学的解析では、イタリアで特定された株とこれより前にインド洋諸島のアウトブレイク時に特定された株との間に高い相同性が示された。ほぼ全例とも症状はかなり軽度で、死亡報告は1例のみであった。

考察:非熱帯地域における今回のCHIKV感染症アウトブレイクは、ある意味予期せぬ事態であり、グローバル化時代における新興感染症の脅威に対する準備と対策の必要性が強く示唆される。

報告企業の意見	今後の対応
2007年7月4日から9月27日までにイタリアにおいてチクングニヤウイルス感染症例205例が集団発生し、グローバル化時代における新興感染症の脅威に対する準備と対策の必要があるとの報告である。	日本赤十字社では、輸血感染症対策として問診時に海外渡航歴の有無を確認し、帰国(入国)後4週間は献血不適としている。国内でチクングニヤ熱が確認されたため、渡航歴確認の徹底を図っている。また、チクングニヤ熱の既往歴がある場合、治癒後6ヵ月間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。

使用上の注意記載状況・その他参考事項等

合成血「日赤」
照射合成血「日赤」
合成血-LR「日赤」
照射合成血-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

Infection with chikungunya virus in Italy: an outbreak in a temperate region

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Lancet 2007; 370: 1840–46

See Comment page 1805

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Summary

Background Chikungunya virus (CHIKV), which is transmitted by *Aedes* spp mosquitoes, has recently caused several outbreaks on islands in the Indian Ocean and on the Indian subcontinent. We report on an outbreak in Italy.

Methods After reports of a large number of cases of febrile illness of unknown origin in two contiguous villages in northeastern Italy, an outbreak investigation was done to identify the primary source of infection and modes of transmission. An active surveillance system was also implemented. The clinical case definition was presentation with fever and joint pain. Blood samples were gathered and analysed by PCR and serological assays to identify the causal agent. Locally captured mosquitoes were also tested by PCR. Phylogenetic analysis of the CHIKV E1 region was done.

Findings Analysis of samples from human beings and from mosquitoes showed that the outbreak was caused by CHIKV. We identified 205 cases of infection with CHIKV between July 4 and Sept 27, 2007. The presumed index case was a man from India who developed symptoms while visiting relatives in one of the villages. Phylogenetic analysis showed a high similarity between the strains found in Italy and those identified during an earlier outbreak on islands in the Indian Ocean. The disease was fairly mild in nearly all cases, with only one reported death.

Interpretation This outbreak of CHIKV disease in a non-tropical area was to some extent unexpected and emphasises the need for preparedness and response to emerging infectious threats in the era of globalisation.

Introduction

Chikungunya virus (CHIKV) is an arthropod-borne virus transmitted to human beings by *Aedes* spp mosquitoes. After the isolation of the virus in Tanzania in 1953,[1] sporadic cases and a number of outbreaks of infection with CHIKV have been reported in several African countries, on the Indian subcontinent, and in southeast Asia.[2] In the past few years, a series of outbreaks have been reported over a large geographical area that includes African islands in the Indian Ocean and the Indian subcontinent. The first of the outbreaks occurred in Kenya in 2004, followed by outbreaks on the Comoros Islands, the island of La Réunion, and other islands in the southwest Indian Ocean in early 2005, and by a large outbreak in India in 2005–06.[3,4] According to the molecular analysis of the strains isolated on islands in the Indian Ocean and in India, the epidemic was caused by a variant of the central/east African genotype of CHIKV.[5]

During the outbreak on islands in the Indian Ocean, a large number of travellers from industrialised countries with a temperate climate became infected with CHIKV and were still infected on returning home.[6–9] In some of these industrialised countries, *Aedes albopictus*—a vector of CHIKV—was introduced a number of years ago and is now widespread,[10] with an especially high population density in Italy.[11] This situation is particularly threatening because it has been suggested that the strain of CHIKV in the Indian Ocean has better adapted to *A albopictus* than it has to other *Aedes* spp.[4] Nonetheless, to date, no outbreaks due to the local transmission of CHIKV have been reported in these countries. Here, we report on a large outbreak of CHIKV infection that occurred in two neighbouring villages in Italy.[12]

Methods

Patients

In July and August, 2007, the local health unit of the province of Ravenna (region of Emilia Romagna, northeastern Italy) detected an unusually high number of cases of febrile illness in Castiglione di Cervia and Castiglione di Ravenna, two small villages divided by a river. In the second week of August, the local health unit implemented an active surveillance system to identify, both prospectively and retrospectively, all individuals with febrile illness, on the basis of reports provided by general practitioners and hospital emergency units. Patient data were collected with a standardised questionnaire and included age, sex, place of residence, countries visited, travel dates, and date of onset of symptoms. In late August, an outbreak investigation was done to identify the agent and the source of the infection and to better understand the dynamics of the epidemic of febrile illness.

Early in the outbreak investigation, infection with CHIKV was suspected because of clinical symptoms and the fact that the first patient with febrile illness was a man from a country affected by an outbreak. Furthermore, the presence of *A albopictus* in the area was known. A case of CHIKV infection was defined as the presence of

high fever (>38·5°C) and joint pain and living in, or having visited, one of the two villages; for this definition, laboratory confirmation was not required. Individuals with fever but no joint pain and those with these symptoms who did not live in the villages or who had not visited them were deemed to be cases only if laboratory confirmation was obtained (ie, positivity to either haemagglutination inhibition or PCR).

Procedures

Blood and serum samples were stored at –80°C before being tested. Samples were tested for antibodies to CHIKV by haemagglutination inhibition and initially confirmed by plaque reduction neutralisation assays in 2 cases who were haemagglutination inhibition-positive; both tests were done at the Istituto Superiore di Sanità, Rome.[11] Thereafter, only the haemagglutination inhibition test was used. Samples were also tested for antibodies to dengue virus and yellow fever, also with the haemagglutination inhibition test.

To detect the presence of viral genomic RNA in human samples, real-time RT-PCR targeting the *nsp1* gene of CHIKV was done. The assay was based on the Qiagen One Step RT-PCR kit, and a 25 µL reaction volume included 3 µL RNA extract (Qiagen Viral RNA Mini kit), 40 ng/µL bovine serum albumin, 400 µmol/L of each dNTP, 600 nmol/L CHIKV sense (tgatcccgactcaaccatcct), 600 nmol/L CHIKV anti-sense (ggcaaacgcagtggtacttcct) primers, and 200 nmol/L probe ChikP (FAM-tccgac-atcatcctccttgctggc-Black Hole Quencher 1). Amplification was done in a Roche Light Cycler (Indianopolis, IN, USA) and involved the following steps: 50°C for 30 min, 95°C for 15 min, and 45 repetitions of 95°C for 15 s then 58°C for 30 s.

PCR was also used to detect CHIKV in specimens of *A albopictus* that were captured locally during the outbreak. Total RNA was extracted from the supernatant of an homogenate of mosquitoes in minimal essential medium, using TRIzol LS (Invitrogen, Carlsbad, CA, USA)). The RNA was retrotranscribed to cDNA with SuperScript II (Invitrogen) and random primers. Two different PCR protocols were used on the same samples: an RT nested PCR[14] and a real-time PCR with Taqman probe.[15]

Two pairs of primers (CHIKV 10264F/CHIKV 11300R and CHIKV 10564F/CHIKV 11081R)[16] were used to

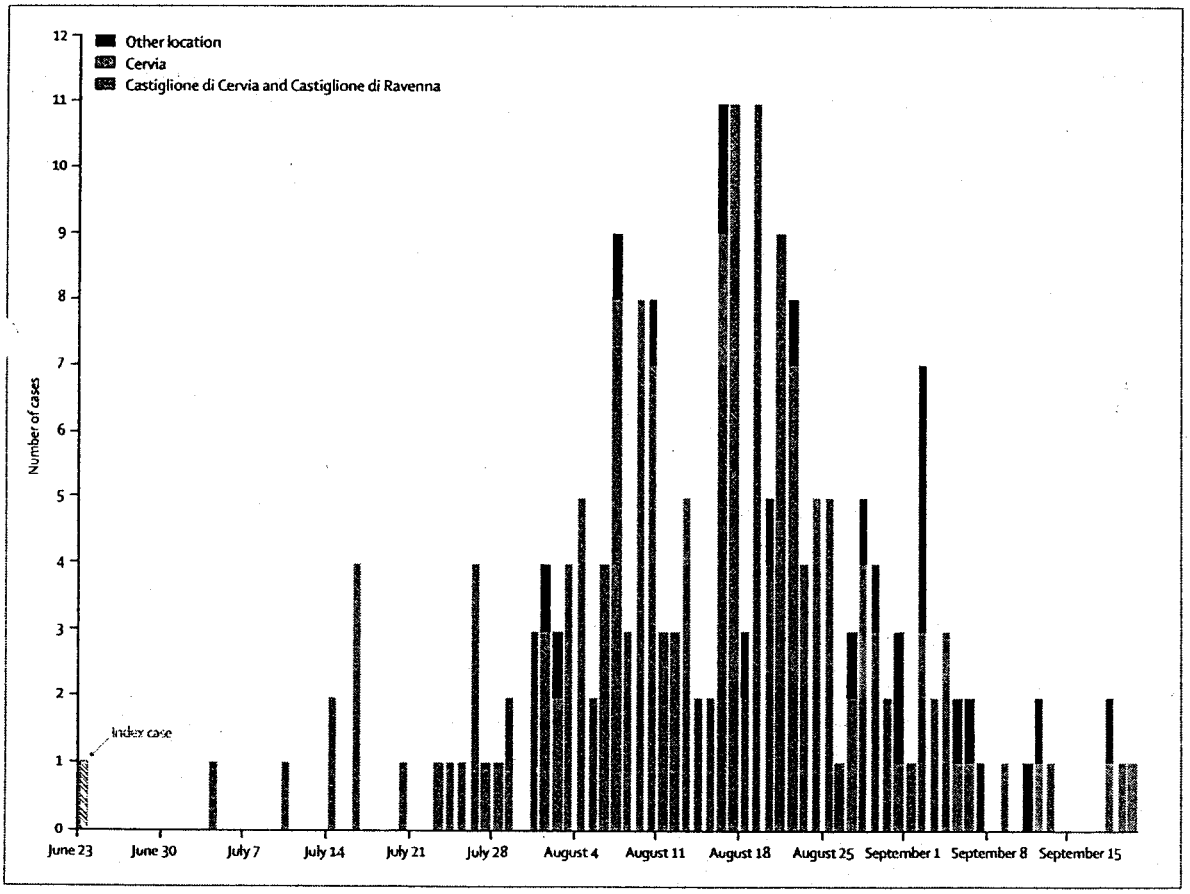

Figure 1: **Epidemic curve**
Distribution of dates of onset of symptoms for CHIKV cases by presumed place of infection (ie, Castiglione di Cervia and Castiglione di Ravenna, Cervia, or other/unknown location).

amplify part of the E1 gene directly from the extracted RNA from RT-PCR-positive samples. Nucleotide sequences were assembled with BioEdit software (version 7·0·9·0) and were aligned with Clustal W software (version 1.6). Phylogenetic analysis based on the available partial E1 gene sequences of CHIKV and tree reconstructions were done with MEGA version 4. For the construction of phylogenetic trees, the neighbour-joining algorithm and the Kimura two-parameter distance model were used. The reliability of the analysis was assessed by a bootstrap test with 1000 replications.

Statistical analysis

In the present analysis, we considered those cases identified between July 4 and Sept 27, 2007. The dates of the onset of symptoms of the cases were plotted to fit the epidemic curve. The frequency distribution of the cases' main characteristics and their signs and symptoms were calculated. Attack rates (both overall and stratified by age and sex) were calculated for the two villages that were affected. Risk ratios (RR) and their 95% CI were also estimated. Age-adjusted attack rates were also calculated separately by sex. The origin and spread of CHIKV cases in the two initially affected villages were mapped. For each case, the address of the individual and the date of the onset of symptoms were entered into Microsoft Access 2003 and linked to the locations on georeferenced maps in the geographic information system (ArcView 8·3, ESRI, Redlands, CA, USA).

Role of the funding source

There was no funding source for this study. All authors had full access to all the data. The corresponding author had final responsibility for the decision to submit for publication.

Results

205 cases of CHIKV infection occurred between July 4 to Sept 27, 2007, in Ravenna (figure 1). There were several waves of cases, with the number peaking in the third week of August. Up to the time of this peak, most cases had occurred in Castiglione di Cervia and Castiglione di Ravenna. Afterwards, and after the first mosquito control measures in the area that was mainly affected had been implemented (on Aug 18), a new wave of cases was observed, most of which occurred outside the two villages.

The distribution of cases by age, sex, residence, and place where the infection was presumably acquired is shown in table 1. The median age was 60 (range 1–95) years; 58 (1–92) years for male cases and 62 (3–95) years for female cases. Most patients reported that they lived in or had visited one of the two villages. The others were scattered throughout the province, although a cluster of 13 cases due to local transmission was reported in Cervia, a town of 8606 inhabitants located about 9 km from the villages. This cluster occurred in a restricted area of the town, near a public gathering place frequented by people coming from—or who had visited—Castiglione di Cervia and who had developed the disease.

	Number of cases (%)
Age (years)	
0–19	12 (6%)
20–39	26 (13%)
40–59	62 (30%)
60–70	78 (38%)
≥80	27 (13%)
Sex	
Male	99 (48%)
Female	106 (52%)
Presumed place of infection	
Castiglione di Cervia or Castiglione di Ravenna	171 (83%)
Cervia	13 (6%)
Other/unknown	21 (10%)
Classification of cases	
Laboratory confirmed	175 (85%)
Clinically defined (untested)	30 (15%)

Table 1: Demographic characteristics of the 205 individuals infected with CHIKV

The first identified case, a man of Indian origin living in Castiglione di Cervia, reported that he had not been abroad during the previous year. However, a relative of his, who had arrived in Italy on June 21 from Kerala, India (an area affected by the CHIKV epidemic), visited the man and became feverish on the afternoon of June 23, when he was in Castiglione di Cervia. A serum sample that had been collected in early September from this man, who was assumed to be the index case, showed high antibody titres against CHIKV (>1:1280). This individual was excluded from the further data analyses.

The spatial-temporal spread of CHIKV in the primarily affected area and the rest of the province is shown in figure 2. After the first cases, which occurred in Castiglione di Cervia, the infection spread both by contiguity, as an expansion of the primary cluster (figure 2 A, B, and C), and by jumping from place to place in both villages, with cases developing more than 2 km away from the primary cluster (figure 2 B and C). Sporadic cases and clusters occurring outside the villages are shown in figure 2 D.

The attack rate was 5·4% in Castiglione di Cervia (115 resident cases out of 2134 inhabitants) and 2·5% in Castiglione di Ravenna (46/1834). The attack rate did not differ between female and male individuals (4·5% of 81 females *vs* 4·0% of 80 males; RR 1·13, 95% CI 0·81–1·57). The rate of attack increased with age: 1·6% of 27 people under 40 years of age, 4·5% of 52 individuals aged 40–59 years, 7·0% of 57 aged 60–79 years, and 8·8% of 25 aged 80 years or older were affected (RR 2·78, 95% CI 1·75–4·39 for the 40–59 years age-group; 4·21,

Figure 2: **Geographical origin and spatial-temporal diffusion of CHIKV cases**
Number of cases in Castiglione di Cervia and Castiglione di Ravenna between days 0–15 (A), between days 0–45 (B), cumulatively (C), and in the province of Ravenna (D).

2·86–6·61 for the 60–79 years age-group; and 5·20, 3·08–8·83 for those aged 80 years or more, all relative to the under 40 years age-group; χ^2 for trend p<0·0001). There was no difference in attack rate between those aged 0–19 years and those aged 20–39 years (1·6% [10/631 individuals] *vs* 1·6% [17/1082]). The age-adjusted attack rates for male and female individuals were much the same (4·2% *vs* 4·1%).

The frequency of clinical symptoms is shown in table 2. All patients presented with high fever (median maximum temperature 39·5°C, 25–75th percentile 39–39·8°C), and most of them had pain in multiple joints. About half the cases developed skin rash, in some cases with itching. Clinical disease was mild and self-limiting in most cases. One 83-year-old man died, although this man had severe underlying conditions.

Laboratory confirmation was obtained for 175 cases: 32 were PCR-positive only; 135 were haemagglutination inhibition-positive only; and eight were positive for both PCR and haemagglutination inhibition. The median time between the onset of symptoms and obtaining positive results was 2 days for PCR (maximum 7 days) and 15 days for haemagglutination inhibition. 30 cases who met the clinical and epidemiological criteria

	Number of cases (%)
Fever*	205 (100%)
Joint paint	199 (97%)
Fatigue	190 (93%)
Skin rash	106 (52%)
Headache	105 (51%)
Muscle pain	94 (46%)
Diarrhoea	48 (23%)
Itching	42 (20%)
Vomiting	40 (19%)
Photophobia	31 (15%)
Conjunctivitis	7 (3%)

*Mandatory in the case definition. †Not mandatory if diagnosis is laboratory confirmed.

Table 2: **Distribution of symptoms**

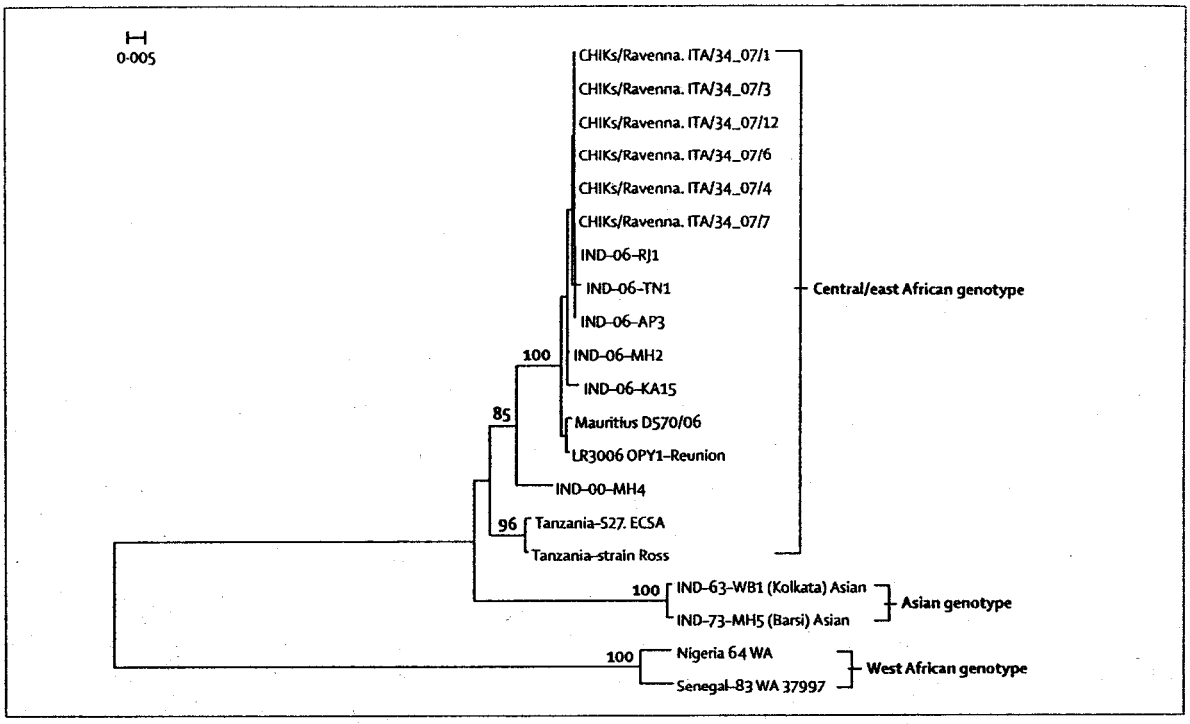

Figure 3: **Phylogenetic analysis of the partial nucleotide sequence (1011 nucleotides) of the E1 gene of CHIKV strains identified in Italy and in different parts of the world**

remained untested because no blood sample was available or because it was inadequate for testing (table 1).

CHIKV sequences were detected by PCR in *A albopictus* mosquitoes captured during the outbreak. Positive results were obtained from pools of 125 mosquitoes from Castiglione di Ravenna and and 90 from Castiglione di Cervia. The results of the phylogenetic analysis are shown in figure 3. Human and mosquito strains clustered with Indian strains, and they contained a change (Ala226Val) in the membrane fusion glycoprotein E1 that had also been found in the Indian Ocean variant of the African genotype of CHIKV.

Discussion

This outbreak of CHIKV infection, outside a tropical country, was probably begun by a man from India, who developed a febrile syndrome 2 days after his arrival in Italy. He had high titres of antibodies against CHIKV at the time of examination (early September) and was probably highly viraemic when visiting his relatives (late June) in the village where the epidemic began. The phylogenetic analysis showed that the strain that caused this outbreak was similar to the strains detected on the Indian subcontinent[5] and that it contained the same mutation found in a variant in the Indian Ocean islands,[7,17] which is thought to be better adapted to *A albopictus* than are other variants. The hypothesis that this variant has a high virus-vector fitness seems to be confirmed by both the successful introduction and rapid spread of the infection from one infected human host and by the further occurrence of other smaller clusters in different localities in the same province yet located several kilometres from the two villages initially affected.

Samples of *A albopictus* mosquitoes from the villages were found to be positive for CHIKV sequences. The high density of the vector at the time of arrival of the index case, as anecdotally reported by villagers, was probably a major determinant of the outbreak. Actually, the population of *A albopictus* was already well established in scattered foci in Ravenna province (an average of >80% positive ovitraps), but had only recently enlarged its peripheral area to include these villages, which might explain the high vector density (ie, before control measures had been implemented). The presence of *A albopictus* in Italy is not surprising. The mosquito was first documented in Genoa (northwestern Italy) in 1990,[18] and the presence of a breeding population was first reported near Padua (northeastern Italy) in 1991.[19] The source of infestation was identified as a warehouse of a tyre retreading company that had imported used tyres infested with mosquito eggs from Georgia, USA.[20] Unfortunately, despite efforts made to control the spread of *A albopictus* mosquitoes, they rapidly colonised almost the entire country,[21,22] showing a high degree of fitness.

The peak of the outbreak occurred during the third week of August, more than 6 weeks after the onset of symptoms in the first locally acquired case, and 8–9 weeks after the onset of symptoms in the presumed

index case. The occurrence of new cases in the initially affected area started to decrease a few days after vector control measures were first implemented. The infection seemed to spread both by contiguity within the initially affected villages and by jumping from place to place within and from the initial outbreak area to the other locations. A small cluster, caused by local transmission, was reported in the town of Cervia, where the infection was probably introduced through population movement from Castiglione, although passive transport of infected mosquitoes cannot be ruled out completely.

The attack rate in Castiglione di Cervia—the most affected village—was 5·4%, much lower than the 34% reported in La Réunion.[4] This difference might be due to early intervention in Italy, although the role of different background vector density or climate-dependent vector behaviour cannot be excluded. Moreover, we cannot rule out under-reporting, which could have occurred if our surveillance system had a low sensitivity in the first month or if there was an excess of asymptomatic cases compared with those found in La Réunion.[23] The attack rates by sex and age, calculated for Castiglione di Cervia and Castiglione di Ravenna, were stably low for people under 40 years of age but tended to increase for older ages, with the highest rates in the oldest group. Whether this trend was due to behavioural factors leading to differential exposure to mosquitoes or to biological factors, implying a different host response with a different proportion of asymptomatic cases, needs to be investigated further.

The clinical course of the disease was fairly mild. The case-fatality rate was less than 0·5%, consistent with the rate of one death per 1000 clinical cases reported in La Réunion.[4] Almost all patients reported joint pain, which was often severe and persistent, and which seems to be strongly indicative of CHIKV disease. Similar findings were reported in La Réunion,[24] whereas a lower proportion of cases with joint pain (78%) was found in Malaysia in 1998.[25] About half the patients presented with skin rash, similar to previous findings.[2]

85% of the cases were confirmed by either serology or PCR. No viral sequences were detected in 31 samples collected more than 7 days after the onset of symptoms, suggesting that the viraemic phase is fairly short, as found in previous reports.[26]

Measures for controlling the population of *A albopictus* were implemented in all areas where cases were reported, beginning on Aug 18. These measures included the use of fast-acting insecticides (synergised pyrethrins) for 3 days consecutively, applied with a truck-mounted atomiser in public spaces and a backpack mist blower in private spaces. Antilarval measures, using formulations of insect growth regulators and *Bacillus thuringiensis* var *israeliensis* were also implemented. House-to-house interventions were done to eliminate breeding places, and community participation was encouraged. For each suspected case of infection, these control measures were done within a radius of 100 m of the individual's residence; for clusters, the control measures were done within a 300-m radius of the most external case. Since Sept 27, 2007—the date at which the present analysis was censored—sporadic cases have continued to occur in Ravenna; two small clusters outside Ravenna (in Cesena and Rimini) have also been identified. Whether transovarial transmission of CHIKV might result in a reappearance of the infection in spring, 2008, is being considered carefully.

The occurrence of an outbreak of CHIKV infection in a country with a temperate climate emphasises that the predicted globalisation of human beings and vectors[4,7] has become a reality. To promptly identify new potential threats that were previously restricted to tropical areas, clinical and diagnostic capacities have to be developed in countries with a temperate climate and in which vectors of exotic diseases already circulate.

Contributors

GR was responsible for the clinical and epidemiological investigation and for writing the manuscript. LN was responsible for laboratory diagnosis and contributed to writing the manuscript. CF, FM, and MGC did laboratory tests on human and mosquito samples, and phylogenetic analysis; PC and MD identified viral sequences in the mosquitoes. MP developed the PCR used in this investigation and contributed to writing the manuscript. RR, GM, and PA were responsible for the entomological investigation and contributed to writing the manuscript. ACF supervised the field activities that were implemented by RA and GS, who also contributed to data analysis. SB was responsible for data management and analysis. AC supervised and coordinated all of the activities and revised the manuscript.

CHIKV study group

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Conflict of interest statement

We declare that we have no conflict of interest.

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別紙様式第2-1

No. 8

医薬品 研究報告 調査報告書

識別番号・報告回数			報告日	第一報入手日	新医薬品等の区分	機構処理欄
				2008. 2. 18	該当なし	

一般的名称	新鮮凍結人血漿	研究報告の公表状況	Flores-Chávez M, Fernández B, Puente S, Torres P, Rodríguez M, Monedero C, Cruz I, Gárate T, Cañavate C. Clin Infect Dis. 2008 Mar 1;46(5):e44-7.	公表国 スペイン
販売名(企業名)	新鮮凍結血漿「日赤」(日本赤十字社) 新鮮凍結血漿-LR「日赤」(日本赤十字社)			

研究報告の概要

○輸血によるシャーガス病:感染受血者および供血者の寄生虫学的、血清学的モニタリング

シャーガス病はラテンアメリカの風土病であるが、人の移動により分布が拡大している。スペインでは、2005年からラテンアメリカ出身の供血者に対して*T. cruzi*抗体検査を実施している。

本報は、血液製剤の輸血によりシャーガス病に感染し、死亡したスペイン人患者の寄生虫学的、血清学的疾患経過、ならびに供血者の調査の報告である。患者は25歳男性で白血病の既往があり、少なくとも176名の供血者由来の血液製剤を輸血されていた。2005年1月(輸血後45日)に原因不明の発熱を発症し、抗菌薬による治療を行った。臍帯血移植後も発熱と神経障害を発症し、多臓器不全で7月上旬に死亡した(輸血後212日)。患者血清中に*T. cruzi* DNAがPCRで確認された。過去の検体を調べたところ、輸血後48日にはDNAが検出されていた。抗体はIFATとELISAで輸血後159日で陽性になり、204日で陰性化していた。輸血された製剤の供血者の血清学検査では、58歳のブラジル出身の女性供血者が抗体陽性であったことが判明した。彼女は2004年12月上旬に供血を行い、血小板製剤が患者に輸血されていた。追加調査時のPCRでは、血中に寄生虫は検出されなかったが、1ヵ月後シャーガス病の精密検査を行った際の血液からはPCRで検出された。

抗体価の動態から、患者はシャーガス病の急性期であったことが示唆された。移植のための免疫抑制状態で、寄生虫が血液脳関門を通過して神経系に感染したことが、CSF検体中の*T. cruzi* DNAから確認された。供血者は無症候の状態であったことから、患者の免疫状態が発症に関連したことが考えられる。複数回輸血患者は、免疫抑制剤治療実施前に、抗*T. cruzi*抗体のスクリーニングを受けるべきである。

報告企業の意見	今後の対応
輸血によりシャーガス病に感染し、死亡したスペイン人患者の寄生虫学的、血清学的疾患経過、ならびに供血者の調査についての報告である。	日本赤十字社は、輸血感染症対策として献血時に海外渡航歴の有無を確認し、帰国(入国)後4週間は献血不適としている。また、シャーガス病の既往がある場合には献血不適としている。日本在住の中南米出身献血者については、国と協議しつつ対応を検討中である。今後も引き続き情報の収集に努める。

使用上の注意記載状況・その他参考事項等

新鮮凍結血漿「日赤」
新鮮凍結血漿-LR「日赤」

血液を介するウイルス、細菌、原虫等の感染
vCJD等の伝播のリスク

22

BRIEF REPORT

Transfusional Chagas Disease: Parasitological and Serological Monitoring of an Infected Recipient and Blood Donor

María Flores-Chávez,[1] Begoña Fernández,[2] Sabino Puente,[3] Pilar Torres,[2] Mercedes Rodríguez,[1] Carolina Monedero,[1] Israel Cruz,[1] Teresa Gárate,[1] and Carmen Cañavate[1]

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Chagas disease is endemic to Latin America, but human migration is extending its distribution. This report describes the parasitological and serological course of disease in a Spanish patient fatally infected via a blood product transfusion, as well as the monitoring of the donor. Before undergoing immunosuppression, multitransfused patients should be screened for anti–*Trypanosoma cruzi* antibodies.

Chagas disease, or American trypanosomiasis, is endemic to Latin America. However, the recent changes in human patterns of migration have prompted the appearance of cases in areas where the vector of the disease is not found [1, 2]. The natural progress of infection involves an acute and a chronic phase. In areas of endemicity both forms are seen, whereas in nonendemic areas, the great majority of infections are diagnosed in the chronic phase, although 70% of infected persons remain asymptomatic. Despite technological advances, there is no reference standard laboratory technique for diagnosing Chagas disease [3]. In the acute phase, parasitological diagnostic methods are the most reliable. However, in the chronic, phase there may be little or no parasitemia, and diagnosis is made mainly on the basis of results of tests for anti–*Trypanosoma cruzi* antibodies. In recent years, Spain has become one of the favorite destination countries for South American emigrants. These citizens achieve a good degree of social integration in Spain, and they often voluntarily and altruistically support blood donation programs. Thus, since 2005, Spanish blood donation legislation has required donors from Latin America to be serologically screened for anti–*T. cruzi* antibodies (Royal Decree 1088/2005) [4]. The present work describes the retrospective laboratory evaluation of a Spanish patient with leukemia who died of Chagas disease contracted via a transfusion with contaminated blood, the retrospective study to identify the source of infection, and the monitoring of the donor.

Methods and materials. Anti–*T. cruzi* antibodies were sought in serum samples collected at different times before the patient's death; these samples were stored at −80°C in the serum library of the Centro Nacional de Microbiología (National Microbiology Center [Madrid]). Parasite DNA was also sought in these samples, in CSF (also collected before death), and in lung, kidney, and liver necropsy samples.

For the retrospective study, serum samples of 176 donors whose blood derivatives had been transfused into the patient were examined. Of these, 168 lived in Madrid (159 were of Spanish origin, 1 was Brazilian, 1 was Ecuadorian, 2 were Colombian, 3 were French, 1 was Polish, and 1 was German), 5 lived in Albacete (southeastern Spain), and 3 lived in Jaén (southern Spain). Samples belonging to all of the Madrid donors were preserved at the serum library of the Centro de Transfusión de Madrid (Madrid Transfusion Center); new samples were collected from the donors living in Albacete and Jaén once they had been traced. Serum and blood samples were collected from the infected blood donor to confirm the results of the retrospective study and to monitor the development of the infection after treatment.

Anti–*T. cruzi* antibodies were detected by the indirect immunofluorescent antibody test (IFAT) and by ELISA with modifications introduced by the Department of Parasitology at the Centro Nacional de Microbiología [5, 6]. *T. cruzi* DNA was detected by PCR with use of oligonucleotides 121–122 and Tcz1–Tcz2, which amplify the variable region of the kinetoplast DNA minicircle (330 bp) and a repetitive sequence of satellite DNA (195 bp), respectively [7, 8]. All assays were performed in duplicate with negative and positive controls.

Results. The Spanish patient was a 25–year-old man who had a history of leukemia [9] that eventually required a cord blood transplant; he received blood derivatives from at least 176 persons who donated blood at different transfusion centers. In January 2005, 45 days after infection onset, the patient was examined for fever of unknown origin. None of the infectious agents that commonly cause this problem in this kind of patient

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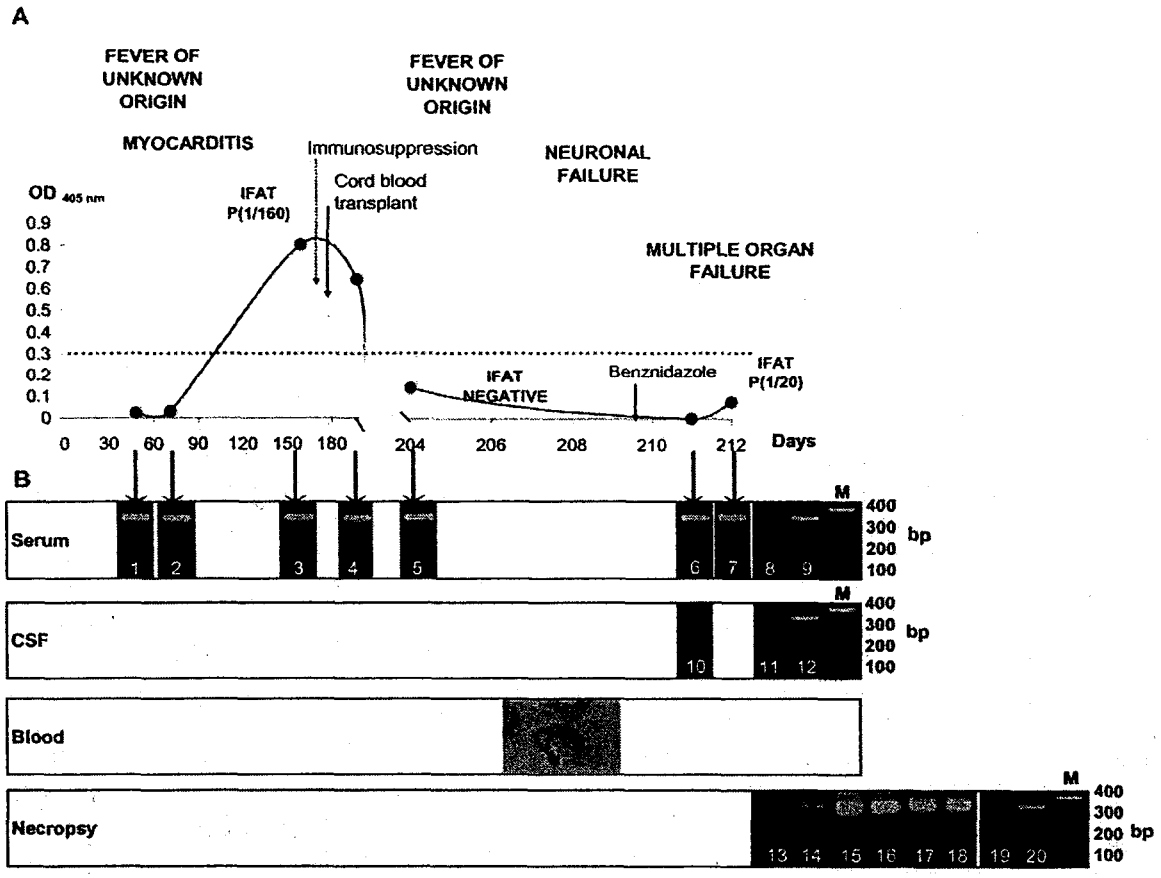

Figure 1. Parasitological and serological changes according to the clinical status of the patient. The day the patient received the platelet concentrate was defined as day 0. *A,* Changes in anti–*Trypanosoma cruzi* antibody levels according to indirect immunofluorescent antibody test (IFAT) and ELISA. The last serum dilution with a positive (P) reaction is shown. *B,* Presence of parasites as determined by microscopy and PCR. *Lanes 8, 11,* and *19,* Negative controls. *Lanes 9, 12,* and *20,* Positive controls. *Lanes 13–14, 15–16,*and *17–18,* Duplicate samples of *T. cruzi* DNA amplified from kidney, liver, and lung tissues, respectively. The PCR results obtained using the oligonucleotides 121–122 confirmed those obtained with Tcz1–Tcz2. *Dotted line,* Threshold; OD, optical density.

(lymphotropic viruses, exanthema-causing viruses, adenoviruses, influenza virus, *Mycoplasma pneumoniae,* or *Toxoplasma gondii,* among others) were detected. After treatment with itraconazole, the symptoms receded, and the patient was assessed and treated in preparation for cord blood transplantation as described by Forés et al. [9]. In the first week of July 2005 (day 211 after infection onset), the Department of Parasitology at the Centro Nacional de Microbiología received several serum and CSF samples obtained from the patient, as well as the supernatants of cell cultures used in the identification of flagellates by microscopy and in diagnostic tests. Microscopy revealed the presence of trypomastigotes, and PCR identified DNA of *T. cruzi,* indicating infection by this pathogen. Tests for anti–*T. cruzi* antibodies, however, yielded negative results.

The patient died of multiorgan failure (day 212 after infection onset), and a retrospective evaluation was undertaken to determine the source of infection. Patient serum samples that were sent to the Centro Nacional de Microbiología for the diagnosis of problems other than Chagas disease and that were preserved at our center's serum library were analyzed by IFAT, ELISA, and PCR (figure 1). PCR showed *T. cruzi* to have first appeared in the patient's serum 48 days after he received a transfusion of platelets. IFAT and ELISA confirmed positive seroconversion on day 159 after infection onset, followed by a negative seroconversion on day 204 after infection onset.

At the same time, the donors whose blood products had been given to the patient were screened for anti–*T. cruzi* antibodies (figure 2*A*). This analysis ruled out all of the donors from Albacete and Jaén and 167 of the donors from Madrid as potential sources of infection. IFAT and ELISA yielded positive results for the remaining Madrid-based donor. This person made a blood donation at the beginning of December 2004 (figure 2*B*); the patient received a concentrate of platelets prepared from this blood (day 0).

The donor was a 58–year-old woman originally from Alto Parnaiba, in the Brazilian state of Maranhão. She was asked to

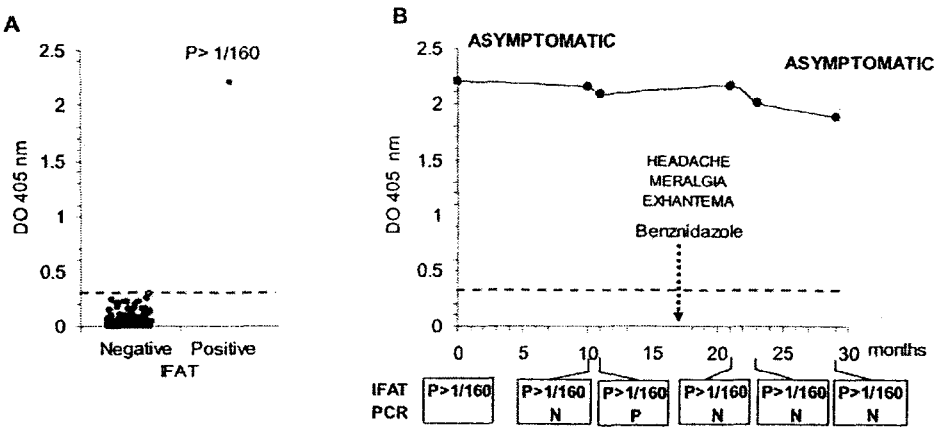

Figure 2. *A,* Determination of anti–*Trypanosoma cruzi* antibodies by indirect immunofluorescent antibody test (IFAT) and ELISA in serum of the different blood donors. The last serum dilution with a positive (P) reaction is shown. *B,* Serological and PCR monitoring of the infected donor. The month in which the infected donor made a blood donation was defined as month 0. *Dotted line,* Threshold. N, negative; OD, optical density.

attend an appointment to confirm the results obtained in the retrospective investigation. At that time, no parasites were detected in her blood by PCR. She was then referred to the Tropical Diseases Unit at the Hospital Carlos III in Madrid, where she underwent a clinical examination, chest radiography, electrocardiography, and echocardiography, all of which yielded normal results. No other signs or symptoms of interest were noted except for constipation, which the donor had experienced for some 8 years (defecation once every 2–3 days). On this occasion (1 month after the first appointment), however, PCR did detect parasites in the blood. In March 2006, treatment with benznidazole (6 mg/kg/day) was begun, but this was suspended after 24 days because of the appearance of intense headaches, meralgia paresthesica of the femorocutaneous nerve, and generalized macular exanthema. No hematologic toxicity was recorded. Following this treatment, test results for blood parasites remained negative, although anti–*T. cruzi* antibodies remained detectable (figure 2*B*).

Discussion. Figure 1 shows that anti–*T. cruzi* antibodies were detectable in the patient only before the start of the immunosuppressive protocol associated with the cord blood transplant (day 159 after infection onset). In the absence of an immune response, the parasites crossed the blood-brain barrier and infected the nervous system. This was confirmed by the presence of *T. cruzi* DNA in the CSF sample. Given the general condition of the patient, treatment with benznidazole had no immediate effect on the parasite load, although IFAT detected a slight increase in the antibody titer (1/20).

The detection of *T. cruzi* in the necropsy samples agrees with the systemic distribution of the parasite and the multiorgan failure that caused the patient's death. The kinetics of the antibody titer can be explained in terms of an acute, recently acquired infection. The detection of *T. cruzi* by PCR since January 2005 (day 48 after infection onset) agrees with the date when the patient received the infected blood products. Thus, the results of the parasitological and serological investigations agree with the patient's clinical signs and symptoms and suggest that he was in the acute phase of Chagas disease. Acute transfusional Chagas disease can last from 1 to 6 months after the entry of the parasite [3].

The discrepant PCR results (1 positive and 1 negative) obtained for the infected donor before benznidazole treatment was begun agree with the low-level parasitemia typical of the chronic phase of *T. cruzi* infection [10]. These results could also be because the first analysis involved a 5-mL blood sample and the second a 10-mL sample. When blood parasite concentrations are low, detection is more likely in larger blood volumes [11]. Similarly, at blood donation units, collecting as much as 450 mL of blood from donors increases the risk of contamination with small numbers of parasites.

Although, for successful blood culturing and artificial xenodiagnosis, it is recommended that blood samples be processed within 4 h of collection to ensure parasite viability [12]; in the present case, the parasites remained viable over the entire platelet conservation period, because the maintenance temperature (22°C; range, 20°C–24°C) is close to that used for culturing *T. cruzi* (25°C–27°C). The recipient's immunodepression caused by his leukemia and the immunosuppression induced before cord blood transplantation appear to have been of maximum importance in the development of the infection, because the parasite caused no appreciable symptoms in the donor. This highlights the role of the host immune system in protection from and the development of infection. In immunodepressed patients, infection may be severe and have fatal consequences. It is therefore recommended that higher-risk organ donors be screened for anti–*T. cruzi* antibodies, as should

multitransfused candidates for transplantation—irrespective of their origin—if they are to undergo immunosuppression protocols.

It should be stressed that before October 2005, Spanish blood donation legislation permanently excluded donors with Chagas disease. It did not, however, contemplate the use of a reliable screening test for the detection of healthy *T. cruzi* carriers. In the present case, the donor did not know of her trypanosome infection status, and no risks were detected during the predonation assessment interview. Her blood donation was therefore accepted in December 2004. In contrast, the current legislation (October 2005) outlines new technical requirements for blood donation [4] and establishes the use of a *T. cruzi* diagnostic assay to assess the eligibility of donors from areas where Chagas disease is endemic, as well as those with risk factors for infection. Under this legislation, the present donor would have been excluded.

In Spain, the supply of blood is a permanent problem, and the Latin American population—~1.5 million residents—has already became an important source of potential donors. A preliminary *T. cruzi* seroprevalence survey of immigrants from areas of endemicity returned positive estimates of close to 1% [13]. Because blood transfusion is the main route for *T. cruzi* transmission in Spain, the new legislation guarantees the quality of blood and blood component transfusions for recipients and allows the inclusion of immigrants from the Americas in the pool of potential blood donors.

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Potential conflicts of interest. All authors: no conflicts.

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医薬品
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化粧品

別紙 3-5

識別番号・報告回数		回	報告日 年　月　日	第一報入手日 2007 年 10 月 11 日	新医薬品等の区分 該当なし	総合機構処理欄
一般的名称			研究報告の公表状況	Blood donor screening for parvovirus B19 in Germany and Austria. Schmidt, M. et al, Transfusion, 47, 1775-1782 (2007).	公表国 ドイツ	
販売名（企業名）						

研究報告の概要	使用上の注意記載状況・その他参考事項等
ドイツ及びオーストリアで 4 年間総計 280 万の献血検体に対して行なわれた B19 ウイルススクリーニングの結果が報告された。測定期間中，2004 年 5 月から 2006 年 1 月におけるウイルス検出頻度が最も高かった。しかし，その B19 DNA 陽性の頻度は 0.274%と低く，B19 ウイルス 1 型のみが検出された。B19 DNA が 10E5 IU/mL を超えた 50 人のドナーからは，初回献血時（T0）から 3 及び 6 ヵ月後の 2 回採血が実施された。詳細な分析の結果，ウイルス価については，T0 時点の中央値が 4.85×10E7 IU/mL から 3 ヵ月後に 4.6×10E2 IU/mL へ有意に減少し，その後 6 ヵ月時点までそのまま推移した検体と，さらに減少した検体が認められた。同時に実施された B19 ウイルス抗体分析では，3 及び 6 ヵ月後の 50 人の全ての検体から，構造蛋白 VP2 に対する中和抗体（IgG）が認められた。従って，この抗体がウイルスを中和していると考えられた。本結果から，本試験に参加中の献血業者の出荷手順を以下のように変更した。 ・10E5 IU/mL を超える高濃度の B19 DNA が検出された献血検体は廃棄とした。しかし，ドナーはその後も献血を行うことができることとした。 ・B19 DNA が 10E5 IU/mL 未満である献血検体は中和抗体を含むため安全と考えられ，輸血された。 ・特殊なリスクを有する患者（小児，妊婦及び免疫が低下した患者）に対しては依然として B19 DNA 陰性の血液製剤が推奨された。	BYL-2008-0301

報告企業の意見	今後の対応
B19 ウイルスの検出頻度は測定の時期及び方法によって，1:260 から 1:50000 まで報告に幅がある。本論文では，4 年間で計 280 万サンプルを測定しており，B19 ウイルスの検出頻度を考慮する上で，信頼性の高いデータを示したと考えられる。また，10E5 IU/ml 以下の B19 ウイルスを含有する検体では相対的に高濃度となる中和抗体が存在し，安全であることが示され，感染リスクを考慮するために重要な情報が提供されていると考えられる。 弊社のポリグロビン N の製造に使用されるミニプール血漿においては，ヒトパルボウイルス B19 に対する NAT を実施しており，10E5 IU/mL 以上が確認された場合は，そのミニプール血漿は製造工程から除去している。現在の科学水準では，ヒトパルボウイルス B19 を確実に不活化する方法は存在しないため，感染リスクを完全に排除することはできないが，伝播の可能性は非常に低いと考える。	ウイルス検出及び安全性に関する閾値に関しては今後とも情報収集に努める。

Blood donor screening for parvovirus B19 in Germany and Austria

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BACKGROUND: Although the main transmission pathway of parvovirus B19 (B19) is typically via the respiratory route, several transfusion-transmitted infections have been reported. To increase blood safety, all blood donations to our blood donor service have been screened by a B19 minipool real-time nucleic acid testing (NAT) since April 2000. Additional customers have been screened since the summer of 2003.

STUDY DESIGN AND METHODS: In total, 2.8 million donations from Germany and Austria were screened for B19 by real-time minipool NAT. A subgroup of 50 B19 DNA–positive donors was screened for B19 immunoglobulin G (IgG) and IgM antibodies and B19 DNA over a 6-month period. Results were compared to those of 100 B19 DNA–negative donors.

RESULTS: Data accumulated over the past 6 years indicate a high incidence period from May 2004 to January 2006. In total, the incidence was 12.7 and 261.5 per 100,000 donations with high virus loads equal to or above 10^5 and below 10^5 IU per mL, respectively. Median virus concentration in the case group was 4.85×10^7 IU per mL at Time Point T0 and was reduced to 4×10^2 IU per mL at the time of the next donation (3 months later). Neutralizing antibodies (VP2) were detected in all donations if virus load was reduced to less than 10^5 IU per mL.

CONCLUSION: The release of B19 DNA–positive blood products with a concentration of less than 10^5 IU per mL is thought to be safe due to the high level of neutralizing VP2 antibodies and is currently examined in a donor recipient infectivity study. In contrast, blood products with a high B19 DNA concentration ($\geq 10^5$ IU/mL), some of which did not contain neutralizing antibodies, were discarded to protect at risk individuals.

Parvovirus B19 (B19) was detected for the first time in 1975 in a blood product from a healthy donor.[1-3] During the onset of B19 infection, virus concentration can increase up to 10^{14} virions per mL.[4-6] Because B19 is a non-lipid-enveloped viral pathogen, inactivation methods like solvent/detergent treatment are ineffective for reduction of virus concentration in plasma. Most infections occur in childhood and result in a mild rash and formation of protective antibodies.[7-13] Infection normally results in seroconversion with neutralizing immunoglobulin G (IgG) antibodies affording lifelong protection from reinfection in most cases.[14] Chronic infection, however, may be associated with a poor antibody response.[15,16]

Screening for B19 DNA by minipool real-time nucleic acid amplification technology (NAT; testing in donor pools up to 96 samples per pool) was introduced into our blood donor screening protocol in 2000. NAT amplification was analyzed in a semiquantitative manner. Blood

ABBREVIATIONS: B19 = parvovirus B19; C_t = cycle threshold.

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products with B19 DNA virus load equal to or higher than 10^5 IU per mL were discarded. In contrast, minipools with B19 DNA virus load below 10^5 IU per mL were not resolved, and all blood products contained were released. In any case, donors were not informed about their B19 infection and were allowed to give subsequent donations.

This study provides results for 4 years of NAT screening, including a case-controlled study for B19 antibodies performed over a 6-month period to monitor the development of structural (VP-1 and VP-2) and nonstructural (NS-1) antibodies.

MATERIALS AND METHODS

Incidence studies

Donations from six different sites were involved in the study. In Germany samples from the German Red Cross Institute Frankfurt (1,732,355 samples, Area 1) and from the German Armed Forces (99,176 samples, Area 2) were included in the study. In contrast, Austrian samples from four test areas including the Medical University of Graz (203,880 samples, Area 3), Austrian Red Cross Institute Klagenfurt (85,811 samples, Area 4), Austrian Red Cross Institute Feldkirch (51,041 samples, Area 5), and Austrian Red Cross Institute of Vienna (626,373 samples, Area 6) were included in the study (Table 1). All donations for the German Red Cross were screened by B19 real-time NAT beginning in April 2000 and in August 2003 for all other institutes. All donations were tested at the GRC Institute in Frankfurt. The screening procedure was not modified during the study period. Donations with B19 virus concentrations of at least 10^5 IU per mL were discarded, whereas minipools that contained donations with a virus load of not more than 10^5 IU per mL were not resolved. All products included in these minipools were designated as being weakly B19 DNA–positive and were released for transfusion. This procedure is in accordance with the requirements of the plasma industry, where the release level per individual donation is 10^5 IU per mL, as well as the German transfusion law, the German authorities (Paul Ehrlich Institute) and the local ethics commission, which approved of this study.

Donor substudy (case-control study)

A group of 50 B19 DNA–positive blood donors with a virus concentration of at least 10^5 IU per mL at the index donation (Time Point T0, high-virus-load group) was analyzed in a prospective study involving two subsequent blood draws (with the first occurring approximately 12 weeks after the index donation), for B19 DNA concentration as well as B19 antibodies. The 50 donors were randomly selected from all B19 DNA–positive donors ($\geq 10^5$ IU/mL) residing in Area 1.

In addition, 100 B19 DNA NAT-negative donors were screened for B19 antibodies as a control group. Both the case and the control groups were comparable with regard to age and sex (Table 1). All donors positive for the presence of B19 DNA ($\geq 10^5$ IU/mL) at the index donation (case group) and 50 randomly selected members of the control group were interviewed by standard questionnaire within 4 weeks after the donation about clinical symptoms of a B19 infection (Table 1).

B19 screening techniques

Routine testing. An aliquot of 100 µL plasma of each blood donation was pooled overnight into minipools containing up to 96 samples per pool. The complete pool of up to 9.6 mL was centrifuged at 58,000 × *g* for 60 minutes at 4°C. Supernatants were discarded and pellets were subjected to nucleic acid extraction with a viral RNA kit (QIAamp, Qiagen, Hilden, Germany). Five-microliter aliquots of the total eluted volume of 75 µL were subjected to polymerase chain reaction (PCR) amplification for B19 DNA. Two positive controls and at least three quantitative standards (10^6, 10^5, and 10^4 IU/mL) were included in each PCR procedure.[17-19]

Resolving of B19 DNA–positive minipools. All samples achieving a positive B19 DNA minipool NAT result with a virus concentration of less than 10^5 IU per mL were released as weakly positive B19 DNA donations without resolving the minipool. In contrast, all

TABLE 1. B19 questionnaire and characteristics of the case and control group*

Characteristic	Group		Significance
	Case	Control	
Total number	50	50	Not done
Men/women	27/23	26/24	0.50
Age (years)	39.0 ± 10.9	44.4 ± 15.1	0.06
Chronic diseases	8/50	12/50	0.23
Tiredness	12/50	11/50	0.50
Joint pains	11/50	9/50	0.40
Neurologic symptoms	1/50	1/50	0.75
Fever, flulike symptoms	1/50	1/50	0.75
Pregnancy	12/23	12/24	0.55
Complications during pregnancy	6/12	2/12	0.10
Disease in childhood			
B19 infection	3/50	2/50	0.50
Rubella	12/50	14/50	0.41
Mumps	10/50	11/50	0.50
Chicken pox	10/50	15/50	0.18
Bordetella pertussis	1/50	3/50	0.31

* Donors of both groups were matched with regard to sex and age and were interviewed about B19-specific clinical symptoms. All women were asked about pregnancies and complications during pregnancies.

minipools that yielded a B19 DNA concentration higher than 10^5 IU per mL were resolved by creating subpools from archive plates. Next the identified B19 DNA–positive samples were discarded and all negative or weakly positive B19 DNA samples included in the minipool were released for transfusion.

NAT. Real-time quantitative amplification of B19 DNA was performed with a CE labeled B19 PCR kit (DRK Baden-Württemberg-Hessen, Frankfurt, Germany) according to the manufacturers' instructions with a thermocycler (ABI PRISM 7000, 7700, 7300, or 7900HT; Applied Biosystems, Foster City, CA). Five microliters of extract was analyzed in a total volume of 25 µL. The assay contains reagents and enzymes for the specific amplification of the VP1-capsid protein gene of B19. Thermal cycling was as follows: 50°C for 2 minutes, 95°C for 15 minutes, 10 cycles of 95°C for 10 seconds and 62°C for 30 seconds, 40 cycles of 93°C for 10 seconds, and 56°C for 40 seconds.

Data analysis was performed with the computer software (sequence detection software, Version 1.6.3, Applied Biosystems). A positive real-time PCR result is reflected by an increase in the fluorescence intensity of a reporter dye. After PCR, the number of PCR cycles necessary to reach a defined fluorescence threshold in each sample was defined as the cycle threshold (C_t). The C_t value is related to the amount of PCR product and therefore to the original amount of target present in the PCR procedure. Low C_t values indicate a high initial target amount and high C_t values indicate the opposite.

Sensitivity and specificity of the DRK B19 PCR kit. Sensitivity was analyzed in accordance with the directive of European Commission 98/79/EC. Probit analysis was done on at least 24 replicates of each dilution from a dilution series containing at least six steps. The calculation was performed on nonlog converted data. Specificity was tested with 200 negative plasma samples. Additionally, the amplification efficiency of different B19 genotypes (Genotype 1, Genotype 2 [Subtype A6], and Genotype 3 [Subtype V9]) was evaluated. Genotype 3 was obtained from a Ghanaian blood donor service.[20-22]

Precautions to prevent B19 DNA cross-contamination. All steps of NAT (pooling, enrichment by centrifugation and extraction, master mix preparation, and amplification) were performed in separate rooms. All rooms were equipped with ultraviolet light and were decontaminated once per week. The daily decontamination procedure included decontamination of all workbenches, pipettes, and centrifuges with a disinfectant (Bacillol Plus, Bode, Hamburg, Germany) and sodium hypochlorite (Roth, Karlsruhe, Germany). All PCR procedures were monitored by the addition of at least six negative controls. PCR procedures were only valid if all negative controls gave a negative test result. All personnel performing nucleic acid extraction and resolution of highly B19 DNA–positive pools have been thoroughly trained to be competent in performing these procedures without cross-contamination.

Screening for B19 antibodies. Samples were screened with two assays for IgM and IgG antibody detection. A parvovirus IgG and IgM assay (recomLine, Mikrogen, Neuried, Germany) was used to analyze antibodies against VP-2, VP-N, VP-1S, VP-2r, VP-C, and NS-1 epitopes. Band intensities were compared with a control band and were scored as –, +/–, 1+, 2+, 3+, or 4+. Additionally all samples were screened with the microtiter plate–based B19 enzyme immunoassay (EIA; Biotrin, Dublin, Ireland) for IgG and IgM antibodies. All antibody assays were performed according to the manufacturers' instructions.

Antibody adsorption. Eight samples with a B19 DNA concentration of more than 10^5 IU per mL and eight samples with a B19 DNA load below 10^5 IU per mL were analyzed for B19 IgG antibodies by use of the recomLine assay. In these samples, virus load was determined by real-time NAT before and after treatment with a protein G column (MAb Trap kit, Amersham, Uppsala, Sweden). One-hundred microliters of each sample was filtered through a protein G column and washed with 5 mL of binding buffer. The flowthrough of the binding step was centrifuged at 58.000 × *g* for 1 hour at 4°C followed by a standard minipool extraction protocol.

B19 sequence analysis. Sequence analysis was performed as described in detail by Hokynar and colleagues.[23] Overlapping amplicons of 1000 bp that spanned the entire protein coding region of the genome were used. Primers (NSofwd and NSirev, NSsfwd and NSorev, p6 and p3, p9 and rtsrev, and rt1 and VP2orev) were used for sequencing plus and minus strands. Amplification products were sequenced directly with a cycle sequencing ready reaction kit (BigDye Terminator, Applied Biosystems, Darmstadt, Germany) and a DNA sequencer (ABI PRISM 310, Applied Biosystems).

Statistical analysis

The sensitivity, standard deviation (SD), and coefficient of variation (CV) of the real-time PCR test were calculated with computer software (Excel 2000, Microsoft Corp., Redmond, WA). For the Probit analysis, another computer program (SPSS 12.0, SPSS, Chicago, IL) was used. Comparison between the case and control groups was calculated with Fisher's exact test or the t test. Statistical significance was assumed if p values were less than 0.05.

RESULTS

Incidence of B19 in different areas

B19 incidence between 2003 and 2006 was demonstrated for six different areas (Fig. 1). There was a high incidence

period of B19 from May 2004 to January 2006 in all screened regions. The highest incidence was found in Areas 4 and 5, although the incidence of B19 DNA–positive donors with a high virus load (B19 DNA concentration $\geq 10^5$ IU/mL) as well as with low B19 DNA virus loads (B19 DNA concentration $<10^5$ IU/mL) did not differ significantly between the areas (Table 2).

Sequence analysis of the 50 B19 NAT–positive blood donors included in the substudy identified only Genotype 1 strains. Sequence analyses of all B19 DNA–positive samples are currently being processed to better understand the genotype distribution in our donor population.

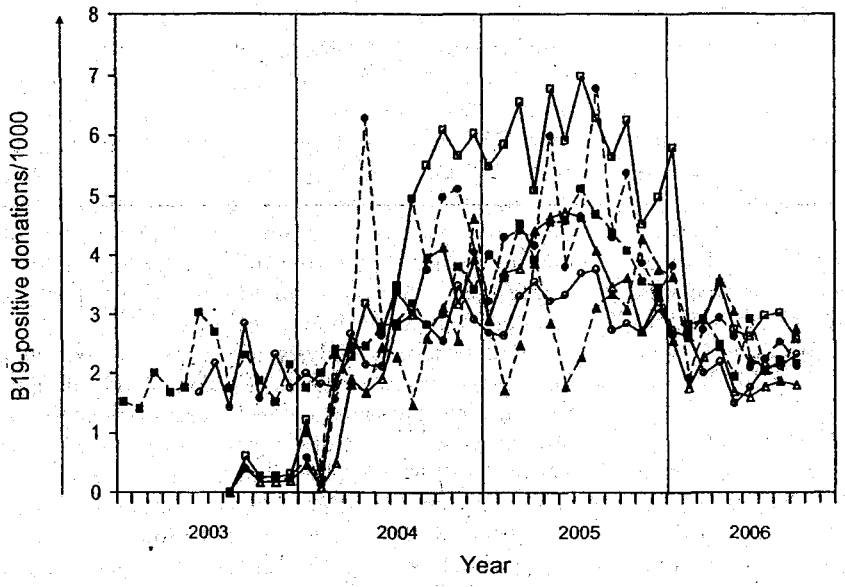

Fig. 1. Incidence of B19 virus infections between 2003 and 2006. Donations were screened for B19 by real-time minipool NAT. Incidence was increased between May 2004 and January 2006, especially in Areas 4 and 5. (■) Area 1 = GRC Institute Frankfurt; (▲) Area 2 = German Armed Forces; (△) Area 3 = Medical University of Graz; (□) Area 4 = Austrian Red Cross Institute Klagenfurt; (●) Area 5 = Austrian Red Cross Institute Feldkirch; and (○) Area 6 = Austrian Red Cross Institute Vienna.

Monitoring of B19 DNA–positive blood donors

In the substudy, two additional donations were taken from 50 B19 DNA–positive multiple-time donors (high-virus-load group) randomly selected from all B19 DNA–positive samples in Test Area 1 to determine B19 DNA concentration and the course of antibody development to B19.

All donors included in the substudy were B19 DNA–positive with a virus load of more than 10^5 IU per mL at the index donation (Time Point T0). The virus load was significantly reduced within 12 weeks from a median of 4.85×10^7 IU per mL (T0; SD) to 4.6×10^2 IU per mL (SD; T1; Fig. 2) and either remained at this level or declined further at Time Point T2. Additional follow-up in a subset of these donors beyond Time Point T2 revealed that B19 DNA concentration was stable around the NAT detection level for up to 1 year (range, 100 and 1500 IU/mL; data not shown). All samples from donors of the case group were below the release level of 10^5 IU per mL at Time Point T1.

B19 antibody levels were investigated with an enzyme-linked immunosorbent assay and a line probe assay. Both commercially available B19 antibody assays gave comparable results for B19 IgM (Table 3) and IgG (Table 4) antibodies. At each time point, IgM antibodies were detected more frequently ($p < 0.05$) in the case group compared with the control group, and antibody titers generally showed an increase from Time Point T0 to Time Point T1 followed by a decrease at Time Point T2. IgM antibodies against the nonstructural protein (NS-1) were not detected at any time point. In contrast, neutralizing IgG antibodies against VP-2 were detected in all samples of the high-virus-load group

TABLE 2. Incidence of B19 virus infections in different areas per 100,000 donations*

	B19 DNA virus load (IU/mL)													
	Area 1		Area 2		Area 3		Area 4		Area 5		Area 6		All	
Year	$>10^5$	$<10^5$	$>10^5$	$<10^5$	$>10^5$	$<10^5$	$>10^5$	$<10^5$	$>10^5$	$<10^5$	$>10^5$	$<10^5$	$>10^5$	$<10^5$
2003	9.3	185.2	1.0	200.3	0.0	19.6	0.0	29.9	0.0	23.9	ND†	ND	6.3	172.0
2004	17.9	254.3	13.6	239.4	9.3	217.0	25.1	340.3	50.0	279.1	11.5	212.9	16.3	247.7
2005	25.0	395.1	12.6	300.6	36.0	345.8	3.9	580.9	6.2	434.7	2.7	295.7	19.4	362.8
2006	5.7	237.1	3.7	209.9	6.0	170.8	0.0	317.1	0.0	255.4	3.6	268.9	4.5	227.6
All	15.0	269.8	9.1	245.0	15.7	221.3	9.3	366.1	17.6	289.7	6.1	259.1	12.7	261.5

* Donations were tested from six different areas in Germany and Austria. Incidence was demonstrated in two groups: 1) donations with high B19 DNA virus load over 10^5 IU/mL and 2) donations with low B19 DNA virus load below 10^5 IU per mL. Incidence increases were observed in all areas in 2004 and 2005 for both groups.
† ND = not done.

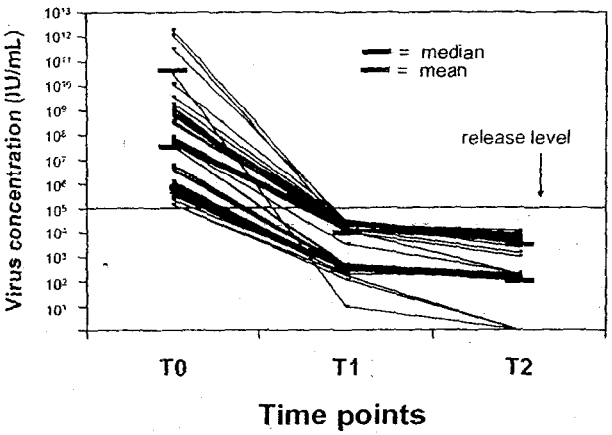

Fig. 2. Virus load during the 6-month study period. All donors of the case group were B19 DNA–positive at the index donation with a value of more than 10^5 IU per mL (highest concentration was 2.1×10^{12} IU/mL). The black bar represents the median virus concentration and the gray bar indicates the mean virus concentration of all donations for each time point. Virus load was significantly reduced from Time Point T0 to Time Point T1. The SDs were 3.5×10^{11}, 1.0×10^4, and 3.5×10^3 for T0, T1, and T2, respectively.

(case group) at Time Point T1 and T2 without any exception. Both antibody assays and titers were significantly higher in the case group compared to the control group. Likewise, antibodies against nonstructural antigens of NS-1 increased up to 92.3 percent from Time Point T0 to Time Point T2.

In one experiment, plasma from donors with B19 DNA concentrations of more than 10^5 IU per mL and with B19 DNA concentrations of less than 10^5 IU per mL was filtered through protein G columns. The viral load was determined before and after IgG absorption. Reduction of the B19 virus concentration was significantly higher in samples with low virus load and high IgG antibodies titers as shown in Table 5. In two of eight samples (viral load, $<10^5$ IU/mL), no virus was detectable after column filtration. In the other six samples, low virus concentrations were detected (mean C_t value, 30.6; virus concentration, <100 IU/mL).

All donors included in the case-control substudy were matched by age and sex and were interviewed with a standard B19 questionnaire about clinical symptoms (Table 1). Typical clinical symptoms for B19 infections such as tiredness, joint pain, or complications between pregnancies did not significantly differ between groups.

TABLE 3. IgM antibodies in the case group and the control group*

	Biotrin EIA	Mikrogen immunoblot					
Group	VP2 (%)	VP-2p (%)	VP-N (%)	VP-1S (%)	VP-2r (%)	VP-C (%)	NS-1 (%)
Case							
T0†	42.9	42.9	42.9	57.1	42.9	35.7	0.0
T1	71.4	71.4	85.7	85.7	50.0	35.7	0.0
T2	23.1	46.2	69.2	69.2	38.5	30.8	0.0
Control							
T0	1.8	9.2	4.6	6.1	1.5	1.5	0.0

* Fifty B19 DNA–positive donors were screened for IgM antibodies by two different assays to detect antibodies against structural (neutralizing) and nonstructural antigens at three time points, and the data were compared to results from 100 B19 DNA–negative blood donors (control group).
† T0 = index donation.

TABLE 4. IgG antibodies in the case and control groups*

	Biotrin EIA	Mikrogen immunoblot					
Group	VP2 (%)	VP-2p (%)	VP-N (%)	VP-1S (%)	VP-2r (%)	VP-C (%)	NS-1 (%)
Case							
T0†	35.7	35.7	28.6	28.6	28.6	21.4	0.0
T1	100	100	100	100	100	85.7	57.1
T2	100	100	100	100	100	76.9	92.3
Control							
T0	74.8	73.3	71.0	68.7	46.6	16.0	14.5

* Fifty B19 DNA–positive donors were screened for IgG antibodies by two different assays to detect antibodies against structural (neutralizing) and nonstructural antigens at three time points, and the data were compared to results from 100 B19 DNA–negative blood donors (control group).
† T0 = index donation.

TABLE 5. IgG adsorption by protein G columns*

Sample B19 virus load (IU/mL)	Total number	Mean C_t Before column absorption	Mean C_t After column absorption	Mikrogen immunoblot mean reactivity ΔC_t	VP-2p	VP-N	VP-1S	VP-2r	VP-C	NS-1
$>10^5$	8	7.5	8.0	0.5	0.4	0.5	0.5	0.8	0.4	0.0
$<10^5$	8	25	30.6	5.6†	2.0	2.9	2.9	3.1	1.8	2.4

* Samples with B19 virus load of more than 10^5 IU per mL and less than 10^6 IU per mL were filtered through protein G columns. The virus load was determined before and after filtering. Additionally, B19 IgG antibodies were analyzed by immunoblots. Band intensities were compared with a control band and were scored as –, +/–, 1+, 2+, 3+, or 4+. For each sample, the mean reactivity is given. Samples with high levels of neutralizing antibodies (higher mean reactivity values) showed significantly higher virus reduction after filtering (higher ΔC_t).
† $p < 0.01$.

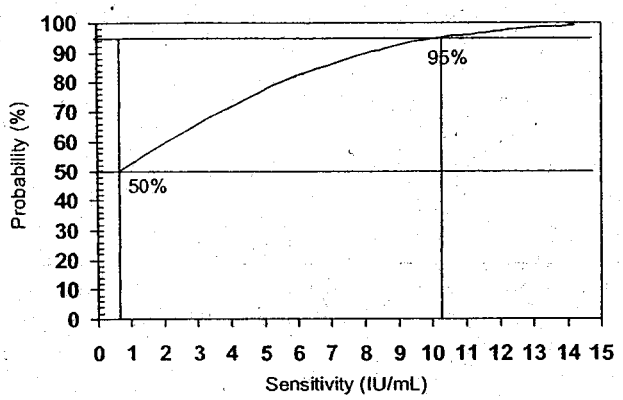

Fig. 3. Sensitivity of B19 NAT kit. The WHO standard (99/800) was diluted into six concentrations. Each standard concentration was tested in 24 replicates. Probit analysis was performed with SPSS Version 12.0 on nonlog converted data. Sensitivity of the minipool NAT was 10.2 IU per mL at 95 percent probability (CI, 7.5 and 18.8 IU/mL) and 0.65 IU per mL at 50 percent probability (CI, –4.1 to 2.47).

Sensitivity and specificity of the B19 PCR kit

As demonstrated in Fig. 3, the 95 percent detection probability of the NAT assay was 10.2 IU per mL (confidence interval [CI], 7.5-18.8 IU/mL) per processed volume. Sensitivity for an individual donation present in a minipool was 982 IU per mL (CI, 724-1811 IU/mL). Specificity was 100 percent as 200 of 200 negative samples gave a negative test result. The amplification efficiency of the DRK B19 PCR kit was comparable for all three genotypes (data not shown). Precision is defined as the degree of scattering within a series of analyses. It is expressed as the SD and the percent CV (%CV). SD and %CV were 0.6, 0.58, and 0.67 and 2.47, 2.37, and 4.20 for intraassay variability, interassay variability, and interbatch variability, respectively.

DISCUSSION

The frequency of B19 viremia in voluntary blood donors has been estimated to range from 1:260 to 1:50,000 and to depend on both the sensitivity of the screening method and the season.[24-26] Here we report results from screening blood donors over a period of more than 4 years with a sensitive real-time NAT method. The mean frequency of DNA-positive blood donors was 274 per 100,000 donations, which was within the range previously reported.[27,28]

Although the incidence of B19 DNA–positive blood products is high, transfusion-transmitted infections have rarely been reported when compared to other transfusion-relevant virus infections like human immunodeficiency virus-1, hepatitis C virus, or hepatitis B virus. This could be explained by the fact that most recipients already have B19 antibodies due to previous infections and that many B19 DNA–positive blood products were also positive for the presence of B19 VP-1 or VP-2 antibodies, resulting in neutralization of the virus. Another possible explanation is that B19 infections were underreported because most recipients get only mild or no clinical symptoms.[29] In the present study, we analyzed the development of anti-B19 and the decrease of B19 DNA in 50 blood donors and compared the data to a control group. In accordance with the literature,[30,31] VP-2 IgG antibodies already existed in the majority (75%) of B19-negative donors (control group). Without exception, all donors in the high-virus-load group (case group) were anti-VP-2 IgG–positive at Time Points T1 and T2. The increase in VP-2 antibodies correlated directly with a significant decrease in B19 virus load. The obvious explanation for this is that the antibodies neutralize the virus.[32]

Although antibodies persist for a long period of time, however, B19 DNA was detectable by real-time NAT for more than 1 year. The question is whether blood products with low levels of B19 DNA and B19 antibodies are infectious. This question is controversial in the literature and is currently being examined by a retrospective donor-recipient study.

Since B19 screening was initiated, the following release procedure was used in our blood donor service. Donations with high B19 DNA concentrations (equal or higher than 10^5 IU/mL) were discarded, but donors were permitted to make subsequent donations and were not informed about their infection. Blood products with B19 DNA concentrations less than 10^5 IU per mL are thought

to contain neutralizing antibodies. Therefore, minipools containing low B19 DNA–positive blood products were not dissolved, and all products contained in those minipools were transfused. The in vitro experiment with a protein G column indicates a significantly higher virus reduction in samples containing low viral loads and high levels of neutralizing antibodies compared to samples containing high viral loads and low B19 antibodies. This observation supports our release procedure, because it suggests that these samples are unlikely to be infectious (Table 5).

Nonetheless, for special-risk patients (immunocompromised patients, young children, or pregnant women), our blood donor service offers B19 DNA–negative blood products. The opportunity to obtain blood testing negative for the presence of B19 DNA has been available since summer 2003. Until now, however, less than 10 B19-negative blood products have been ordered, which demonstrates that physicians are relatively unaware of this infection.

In summary, all blood products have been screened for B19 by a real-time minipool PCR since 2000. A high-incidence period was observed between May 2004 and January 2006. Transfusion of blood products with a low virus concentration seems to be safe because of the coexistence of neutralizing antibodies, whereas blood products with high virus concentrations may pose a risk for transfusion recipients. Therefore, these donations were discarded at our blood donor service. Transfusion-transmitted B19 infections might be underreported and should be examined in donor-recipient studies.

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別紙様式第2-1

No. 11

医薬品 研究報告 調査報告書

識別番号・報告回数			報告日	第一報入手日	新医薬品等の区分	機構処理欄
				2008. 1. 21	該当なし	

一般的名称	人免疫グロブリン	研究報告の公表状況	Modrof J, Berting A, Tille B, Klotz A, Forstner C, Rieger S, Aberham C, Gessner M, Kreil TR. Transfusion. 2008 Jan;48(1):178-86. Epub 2007 Sep 27.	公表国 オーストリア
販売名(企業名)	人免疫グロブリン「日赤」(日本赤十字社)			

研究報告の概要

○血漿および静注用免疫グロブリン製剤によるヒトパルボウイルスB19の中和

背景:ヒトパルボウイルスB19(B19V)は広く蔓延する病原体であり、血漿由来製剤原料血漿プールは、B19V抗体(B19V免疫グロブリンG[IgG])を含有することが示されている。

試験デザインおよび方法:巨核芽球細胞株UT7/Epo-S1はB19V 遺伝子型1に感染し、また、本試験において、免疫組織学的方法、ウエスタンブロット法、B19V特異的mRNAのRT-PCR法を用いて示したとおり、最近発見された遺伝子型2にも感染する。感染UT7/Epo-S1細胞のB19V RT-PCR解析に基づき、感染実験を確立し、B19V中和検査を実施した。B19V IgG力価に関係したB19V中和抗体の役割を検討するため、製造血漿プール1000以上について酵素免疫測定法による検査を実施した。

結果:血漿プールは、B19V IgG力価:平均33±9IU/mL(最小値11IU/mL)を含有することが判明した。これらの11IU/mLのB19V IgGは、B19V遺伝子型1の感染性を4.6 log、遺伝子型2の感染性を3.9 log以上を中和した。このため、このようなプール由来の10%静注用免疫グロブリン製剤(IVIG)は、さらに高いB19V中和活性を含有することが分かった。

結論:分画用血漿プールにおけるB19遺伝子型1、2中和活性の高さは、当該プールのB19V IgG力価が一貫して高いために備わった特徴であることが示された。検討した10%IVIG製剤において、B19V IgGの中和活性を維持することが示された。

報告企業の意見	今後の対応
血漿由来製剤原料血漿プールにおけるヒトパルボウイルスB19中和活性の高さは、当該プールのB19V IgG力価が一貫して高いために備わった特徴であり、検討した10%IVIG製剤において、B19V IgGの中和活性を維持することが示されたとの報告である。	本製剤は現在製造・供給しておらず、当面特別な対応を必要としない。

使用上の注意記載状況・その他参考事項等

人免疫グロブリン「日赤」

ウイルス等の感染性

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