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

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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]

[1]Servicio de Parasitología, Centro Nacional de Microbiología, Instituto de Salud Carlos III, and [2]Centro de Transfusión de la Comunidad de Madrid, and [3]Unidad de Enfermedades Tropicales, Hospital Carlos III, Madrid, Spain

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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Reprints or correspondence: Dr. Carmen Cañavate, Servicio de Parasitología, Centro Nacional de Microbiología, Instituto de Salud Carlos III, Ctra. Pozuelo-Majadahonda, Km 2 28220 Majadahonda, Madrid, Spain (ccanave@isciii.es).

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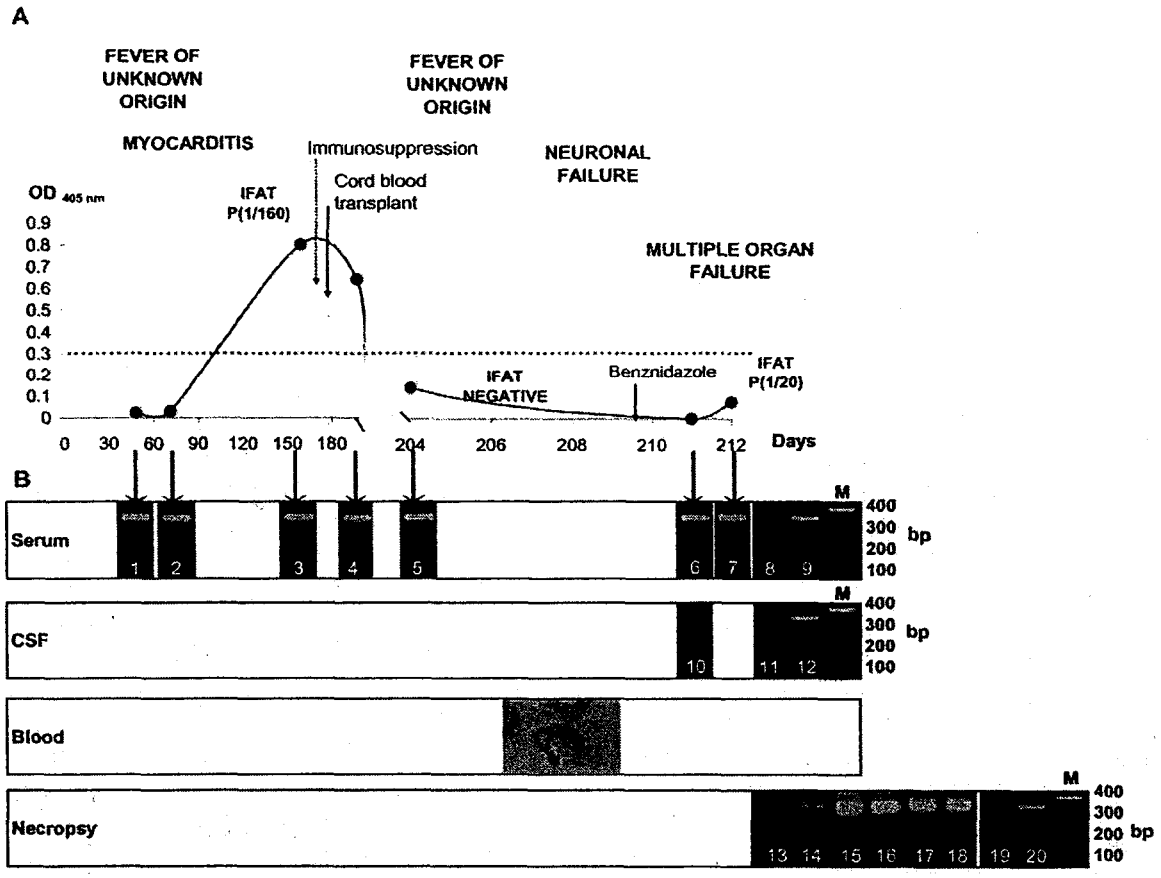

Figure 1. Parasitological and serological changes according to the clinical status of the patient. The day the patient received the platelet concentrate was defined as day 0. *A,* Changes in anti–*Trypanosoma cruzi* antibody levels according to indirect immunofluorescent antibody test (IFAT) and ELISA. 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

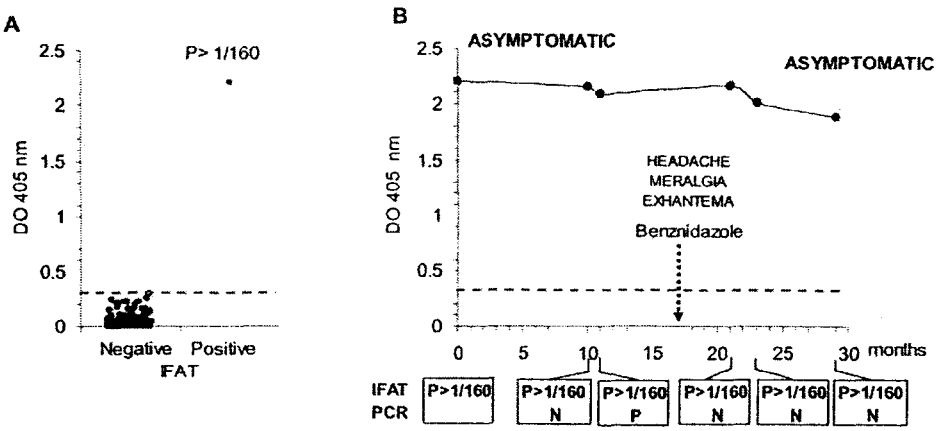

Figure 2. *A,* Determination of anti–*Trypanosoma cruzi* antibodies by indirect immunofluorescent antibody test (IFAT) and ELISA in serum of the different blood donors. The last serum dilution with a positive (P) reaction is shown. *B,* Serological and PCR monitoring of the infected donor. The month 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.

Acknowledgments

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Potential conflicts of interest. All authors: no conflicts.

References

1. Gascon J, Grupo de Trabajo del Taller Enfermedad de Chagas Importada: Un nuevoreto de salud publica? Diagnosis and treatment of imported Chagas disease [in Spanish]. Med Clin (Barc) **2005**; 125:230–5.
2. Wendel S, Gonzaga AL. Chagas' disease and blood transfusion: a New World problem? Vox Sang **1993**; 64:1–12.
3. World Health Organization. Control of Chagas disease. Geneva: World Health Organization Press, **2002**:1–124.
4. Royal Decree 1088/2005. Technical requirements and minimum conditions of the blood donation and of the transfusion centers and services [in Spanish]. Official Spanish Gazette **2005**; 225:31288–304.
5. Camargo ME. Fluorescent antibody test for the serodiagnosis of American trypanosomiasis: technical modification employing preserved culture forms of *Trypanosoma cruzi* in a slide test. Rev Inst Med Trop Sao Paulo **1966**; 8:227–35.
6. Scott P, Pearce E, Natovitz P, Sher A. Vaccination against cutaneous leishmaniasis in a murine model. I. Induction of protective immunity with a soluble extract of promastigotes. J Immunol **1987**; 139:221–7.
7. Wincker P, Britto C, Pereira JB, Cardoso MA, Oelemann W, Morel CM. Use of a simplified polymerase chain reaction procedure to detect *Trypanosoma cruzi* in blood samples from chronic chagasic patients in a rural endemic area. Am J Trop Med Hyg **1994**; 51:771–7.
8. Moser DR, Kirchhoff LV, Donelson JE. Detection of *Trypanosoma cruzi* by DNA amplification using the polymerase chain reaction. J Clin Microbiol **1989**; 27:1477–82.
9. Forés R, Sanjuan I, Portero F, et al. Chagas disease in a recipient of cord blood transplantation. Bone Marrow Transplant **2007**; 39:127–8.
10. Castro C, Prata A. Absence of both circadian rhythm and *Trypanosoma cruzi* periodicity with xenodiagnosis in chronic chagasic individuals. Rev Soc Bras Med Trop **2000**; 33:427–30.
11. Junqueira AC, Chiari E, Wincker P. Comparison of the polymerase chain reaction with two classical parasitological methods for the diagnosis of Chagas disease in an endemic region of north-eastern Brazil. Trans R Soc Trop Med Hyg **1996**; 90:129–32.
12. Castro C, Santos MC, Silveira CA. Comparative study between artificial xenodiagnosis performed immediately and four hours after venous punch. Rev Soc Bras Med Trop **2004**; 37:128–30.
13. Castro E. Blood transfusion and Chagas disease: initiatives in Spanish transfusion centers [in Spanish]. Enferm Emerg **2006**; 8 (Suppl. 1): 48–50.

別紙様式第 2-1

医薬品
医薬部外品　研究報告　調査報告書
化粧品

別紙 3-5

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

Michael Schmidt, Anna Themann, Camilla Drexler, Michaela Bayer, Gerhard Lanzer, Eva Menichetti, Sigrid Lechner, Dietmar Wessin, Barbara Prokoph, Jean-Pierre Allain, Erhard Seifried, and Michael Kai Hourfar

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.

From the German Red Cross, Institute Frankfurt, Frankfurt, Germany; the Department of Blood Group Serology and Transfusion Medicine and Immunohematology, Medical University of Graz, Graz; the Austrian Red Cross, Institute Vienna, Vienna; the Austrian Red Cross, Institute Klagenfurt, Klagenfurt; the Austrian Red Cross, Institute Feldkirch, Feldkirch, Austria; the German Military Forces, Institute for Transfusion Medicine, Koblenz, Germany; and the University of Cambridge, Cambridge, United Kingdom.

Address reprint requests to: Michael Schmidt, MD, Institute of Transfusion Medicine and Immunohematology, Johann Wolfgang Goethe University, German Red Cross, Sandhofstrasse 1, 60528 Frankfurt am Main, Germany; e-mail: mschmidt@blutspende.de.

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TRANSFUSION 2007;47:1775-1782.

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.