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研究報告の概要

ヒトパルボウイルスB19 DNAは、しばしば血漿由来凝固因子製剤に検出される。加えてB19感染の伝播が観察され、製造工程中に日常的に行われるウイルスの不活化/除去の工程にもかかわらず、感染性ウイルスが存在していることを示している。最近、ヒトパルボウイルスB19の分類が3つの異なる遺伝型に分かれることが確認された。これまで、凝固因子製剤の遺伝子型2による汚染の情報はない。このため、我々はPCRにより、遺伝子型1及び2について、202の異なる凝固因子製剤のロットを調査した。最近3年間に投与された13の異なる製品の181のロットについて分析を行い、1980年代初めまで使用されたウイルス不活化処理のされていなかった21ロット（8製品）と比較した。遺伝子型1 DNAが、現在投与されているロットの77/181（42.5%）に、以前に使用されたロットの17/21（81%）に検出された。遺伝子型2 DNAは、5/202（2.5%）に見出され、その5ロット全てが、遺伝子型1 DNAにも汚染されていた。遺伝子型2 DNAが見出された5ロットは、血液凝固第VIII因子製剤の5ロットで、現在使用されているロットで2ロット、1980年代初めまで使用されていたロットで3ロットが含まれていた。
DNA配列分析は、PCRで2重に陽性であった製剤は、典型的な遺伝子型1と遺伝子型2のDNAを含んでいることを示していた。遺伝子型2は遺伝子型1と類似の疾患スペクトラムを起こすように見えることから、現在プール血漿に広く適用されている遺伝子型1に加えてNATでの遺伝子型2の同時検出によって、血液製剤の安全性のレベルを引き上げることができるであろう。

使用上の注意記載状況・その他参考事項等

代表として献血ヴェノグロブリン-IH ヨシトミの記載を示す。
2. 重要な基本的注意
(1)略
1) 血漿分画製剤の現在の製造工程では、ヒトパルボウイルスB19等のウイルスを完全に不活化・除去することが困難であるため、本剤の投与によりその感染の可能性を否定できないので、投与後の経過を十分に観察すること。
3. 略
4. 略
5. 略
6. 妊婦，産婦，授乳婦等への投与妊婦又は妊娠している可能性のある婦人には、治療上の有益性が危険性を上回ると判断される場合にのみ投与すること。〔妊娠中の投与に関する安全性は確立していない。本剤の投与によりヒトパルボウイルスB19の感染の可能性を否定できない。感染した場合には胎児への障害（流産，胎児水腫，胎児死亡）が起こる可能性がある。〕

報告企業の意見

ヒトパルボウイルスB19の変異型である遺伝型2が血液凝固第VIII因子製剤から検出された報告である。
弊社が最終製剤の試験に用いているキットは医学生物学研究所製「スマイテストパルボウイルスB19遺伝子定性キット」であり、このキットは、パルボウイルスB19遺伝型2についても検出可能であることを確認している。万一、原料血漿にパルボウイルスB19遺伝型2が混入したとしても、CPVをモデルウイルスとしたウイルスバリデーション試験成績およびパルボウイルスB19を用いた不活化・除去試験結果から、本剤の製造工程において十分に不活化・除去されると考えている。

今後の対応

本報告は本剤の安全性に影響を与えないと考えるので、特段の措置はとらない。

Contamination of coagulation factor concentrates with human parvovirus B19 genotype 1 and 2

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Summary

Human parvovirus B19 (B19) DNA has frequently been detected in plasma-derived coagulation factor concentrates. Furthermore, transmission of B19 infection was observed, indicating presence of the infectious virus despite routine viral inactivation/removal procedures during the manufacturing process. Recently, human parvovirus DNA isolates, variant from B19, have been identified resulting in classification of B19 virus into three distinct genotypes, with all viruses previously classified as B19 belonging to genotype 1. So far, there is no information available on contamination of clotting factor concentrates with genotype 2. Therefore, we analysed 202 different factor concentrate lots for genotype 1 and 2 DNA by PCR. Analysis of one hundred eighty-one lots representing 13 different products, administered over the last three years, was compared to 21 lots (8 products) used until the early 1980s which had not been treated by viral inactivation procedures. Genotype 1 DNA was detected in 77/181 (42.5%) currently administered lots, and 17/21 (81%) previously used lots. The level of genotype 1 DNA contamination was similar in currently and previously administered concentrates. Genotype 2 DNA was found in 5/202 (2.5%) lots, all of which were co-contaminated with genotype 1 DNA. DNA sequence analysis showed that the PCR-double positive concentrates contained typical genotype 1 and genotype 2 DNA. Because genotype 2 appears to cause a similar spectrum of diseases as genotype 1, simultaneous detection of genotype 2 by nucleic acid amplification testing (NAT), now widely applied to plasma pools for genotype 1, would give an added level of safety to blood products.

Keywords

Human parvovirus, genotype 2, coagulation factor concentrates

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Introduction

Human parvovirus B19 (B19) DNA has frequently been detected in plasma-derived coagulation factor concentrates (1-5). Transmission of B19 infection by derivatives produced from pooled plasma has been reported thus indicating the presence of the infectious virus despite routine viral inactivation/removal procedures during the manufacturing process (6-12). However, the effectiveness against B19 of the current inactivation procedures is unclear due to physical robustness of the nonenveloped virus.

Recently, it has been shown that the genetic diversity of B19 virus is higher than previously expected (13-15; for review 16). Therefore, the species B19 is now subdivided into three different genotypes, with all viruses previously classified as B19 belonging to genotype 1. Until now, genotype 2 DNA has been detected in several European countries including Germany, and the United States (14, 17, 18), whereas detection of B19 genotype 3 was mainly limited to France (15). According to current data, genotype 2 and genotype 3 viral infections cause the same spectrum of illnesses as "classical" B19 infections (15). Given the relatively high homology between the viral proteins, sero-

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logical cross-reaction and a certain degree of cross-protection between the three genotypes could be expected, at least in immunocompetent individuals.

Since there is no information on the frequency of contamination with B19 genotype 2, we investigated, by nested polymerase chain reaction, 202 lots of coagulation factor concentrates representing 21 commercially available products for both genotype 2 and genotype 1 DNA. One hundred and eighty-one lots (13 different products) were currently used concentrates administered during the last three years and 21 lots (8 different products) were formerly used concentrates administered up to the beginning of the 1980s and not virally inactivated. In case of a positive genotype 2 result, DNA sequence analysis was performed. The viral load of genotype 1 DNA in currently and formerly administered concentrates was quantitatively measured by real-time polymerase chain reaction.

Table 1: Detection of parvovirus B19 genotype 1 and genotype 2 DNA in currently administered coagulation factor concentrates.

Coagulation factor	Product	Virus inactivation	IU[1]	No. of lots tested	PCR results: No. of positive lots (%) Genotype 1	Genotype 2
Factor VIII	A	S/D & dry heat 80 °C 72 h	1000	35	7	0
			500	7	4	0
				Σ 42	11	0
	B	Tween 80 & vapour heat 60 °C 10 h	1000	16	12	0
			500	8	4	0
			250	4	0	0
				Σ 28	16	0
	C	S/D & dry heat 100 °C 0.5 h	1000	25	20	2*
			500	2	0	0
				Σ 27	20	2
	D	S/D	1000	11	3	0
	E	Pasteurisation 60 °C 10 h	1000	7	1	0
			500	2	0	0
				Σ 9	1	0
	F	Pasteurisation 60 °C 10 h	1000	10	7	0
	G	S/D & dry heat 80 °C 72 h	1000	1	1	0
	H	S/D & dry heat 100 °C 0.5 h	250	1	1	0
				Total 129	60 (46.5%)	2 (1.6%)
Factor IX	I	Tween 80 & vapour heat 60 °C 10 h, 80 °C 1 h	1000	14	5	0
			600	4	2	0
				Σ 18	7	0
	J	S/D & nanofiltration	1000	10	4	0
			500	3	1	0
				Σ 13	5	0
	K	S/D	500	2	0	0
				Total 33	12 (36.4%)	0
Factor VII	L	Vapour heat 60 °C 10 h, 80 °C 1 h	500	2	0	0
Act. prothrombin complex concentrate	M	Vapour heat 60 °C 10 h, 80 °C 1 h	1000	8	3	0
			500	9	2	0
				Total 17	5	0
All products				Total 181	77 (42.5%)	2 (1.1%)

[1] IU = International Units coagulation factor per vial
* Genotype 2 DNA positive lots referred to as C_1 and C_2

Materials and methods

Coagulation factor concentrates

In total, 202 lots of 21 commercially available plasma-derived coagulation factor concentrates were investigated. One hundred and eighty-one lots (13 different products) were currently available concentrates, administered during the last three years (specimens collected between October 18, 2000 and February 28, 2003), compared to 21 lots (8 different products) taken from formerly used concentrates, administered until the beginning of the eighties and not virally inactivated. Details of the investigated coagulation factor concentrates are given in Table 1 and Table 2.

DNA isolation and polymerase chain reaction

DNA was prepared from 200 µl of reconstituted factor by spin column procedure (QIAamp DNA® Mini Kit, Qiagen, Hilden, Germany) according to the instructions of the manufacturer. Nested polymerase chain reaction (PCR) for detection of B19 genotype 1 DNA was performed as described previously (19). The assay was found to reliably detect the presence of 2 genome equivalents per reaction. PCR was specific for genotype 1 DNA. Amplification of genotype 2 DNA was performed using the primers described by Hokynar et al. (14). PCR was carried out in 50 µl volumes with the following concentration of reagents: 250 µM of each deoxynucleoside triphosphate (Ultrapure dNTPs, Amersham Biosciences, Freiburg, Germany), 25 pmol of each primer (Sigma-Genosys, Steinheim, Germany), 5 µl 10× PCR-buffer (Expand High Fidelity PCR System, Roche Diagnostics, Mannheim, Germany), 2 mM $MgCl_2$ (Roche Diagnostics) and 1.75 U DNA polymerase (Expand High Fidelity PCR System, Roche), and 5 µl of DNA preparation. From first round reaction mixture, 5 µL were transferred to the second round reaction mixture containing the same constituents as the first round mix, except for the nested primers. Amplification was as follows: 95 °C for 5 min followed by 35 cycles each consisting of 94°C for 10 s, 50°C for 10 s, and 72°C for 20 s (T3 Thermocycler, Biometra®, Göttingen, Germany). A final elongation step followed for 3 min at 72°C. Identical conditions were used for the first and second round amplification. The assay accurately detects 2 genome equivalents per sample, determined as mentioned below. Figure 1 illustrates the positions of the amplified regions in the B19 genome.

10 µl of the second-round PCR mixture were analysed by electrophoresis on agarose composite minigels of 1.5%NuSieve® GTG® [FMC]/0.5%SeaKem® LE [FMC] (Cambrex, supplied by Biozym, Hessisch Oldendorf, Germany). Amplified products were visualised by ethidium bromide staining and UV illumination. Positive and negative controls were included in every run. For negative control, all PCR reagents and sterile bidistilled water instead of the sample was used. Strict precautions to avoid contaminations were taken.

DNA sequence analysis

For DNA sequence analysis, half of the genome was amplified by nested PCR using genotype-specific oligonucleotide primers (Fig. 1). For sequencing of genotype 1 DNA, all four nested primer pairs described by Hemauer et al. (20; amplification regions: NS1-C, ΔV, VP1/VP2 and VPC) were used. Additionally, primers for amplification of the genome region between amplification regions VP1/VP2 and VPC (region VPint) were used: outer forward 5′-ACAATGCCAGTGGAAAGGAG-3′ (nucleotide (nt) 3318-3337; all positions according to B19 genotype 1 strain Au, GenBank accession no. M13178) (21); outer reverse 5′-CCCAGGGCGTAAGGA-

Table 2: Detection of parvovirus B19 genotype 1 and genotype 2 DNA in coagulation factor concentrates administered until the beginning of the 1980s.

Coagulation factor	Product	IU[§]	No. of lots tested	PCR results: No. of positive lots (%)	
				Genotype 1	Genotype 2
Factor VIII	a	250-1100	8	6	0
	b	250 / 500	4	4	1*
	c	250 / 500 / 1000	4	2	0
	d	1000	1	1	1
	e	250	1	1	0
	f	1000	1	1	0
	g	500	1	1	1
	h	1000	1	1	0
			Σ 21	17 (81%)	3 (14%)

§ IU = International Units coagulation factor per vial
* Lot contains 500 IU

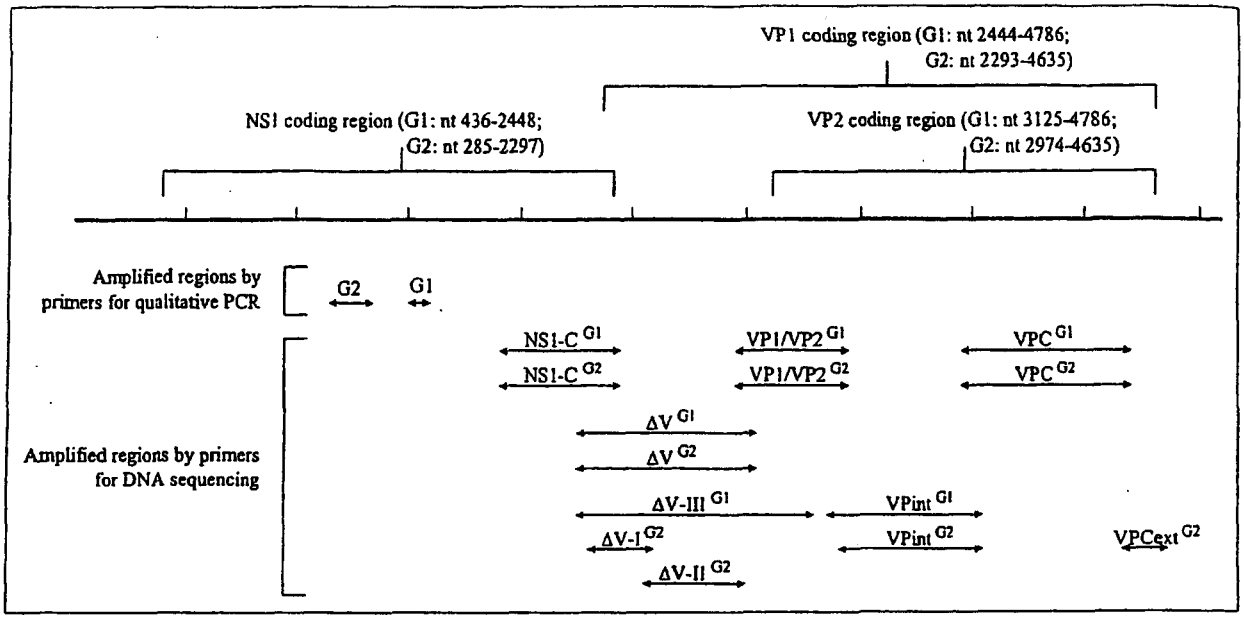

Figure 1: Schematic representation of the amplified regions of the parvovirus B19 genome. The reading frames of the viral proteins NS1, VP1 and VP2 are indicated in the upper part of the figure. Numbering of the nucleotides of B19 genotype 1 (G1) according to the genotype 1 strain Au (GenBank accession no. M13178); numbering of the nucleotides of genotype 2 (G2) according to strain LaLi (GenBank accession no. AY044266). The genome regions amplified by nested PCR using genotype 1 (G1) and genotype 2 (G2) -specific primers are shown in the lower part of the figure. G1- and G2-specific primers used for qualitative PCR were previously described (14, 19); G1- and G2-specific primers for DNA sequencing as mentioned in *material and methods*.

TATT-3′ (nt 4117-4099); nested forward 5′-AAGGTTTGCAC-CATCAGTCC-3′ (nt 3341-3360); nested reverse 5′-TTAAG-GCTTTTCCAGCTCCA-3′ (nt 4064-4045). In cases where no PCR product was obtained by ΔV-specific primers, the following primer set was used (amplification region ΔV-III): outer forward 5′-CTACACACCTTTGGCAGACC-3′ (nt 2151-2170); outer reverse 5′-GGACTGATGGTGCAAACCTT-3′ (nt 3360-3341); nested forward 5′-TTTACCTGTGTGTTGTGTGCAA-3′ (nt 2223-2244); nested reverse 5′-CTGCGGGAGAAAA-CACCTTA-3′ (nt 3305-3286).

For amplification of genotype 2 DNA the sequences of the primers used for the genotype 1 amplification regions NS1-C, ΔV, VP1/VP2 and VPC were modified according to the sequence of the genotype 2 strain LaLi (14; GenBank accession no. AY044266). Primers for amplification of the genome region between amplification region VP1/VP2 and VPC (amplification region VPint) were as follows: outer forward 5′-CAGTG-GAAAAGAGGCAAAGG-3′ (nt 3174-3193; nucleotide positions according to genotype 2 strain LaLi; note that homologous genome regions of genotype 1 and 2 are not congruently numbered); outer reverse 5′-CCAGTGATGGTATGGCTGTG-3′ (nt 3993-3974); nested forward 5′-CATAATGGGCTACTCAA-CACCA-3′ (nt 3210-3231); nested reverse 5′-GCGCC-TGTATTGGAAGTGTC-3′ (nt 3899-3880). When the modified primers failed to amplify the region ΔV the following primer sets were used: amplification region ΔV-I: outer forward 5′-ATTGCCTGTTTGTTGTGTGC-3′ (nt 2072-2091); outer reverse 5′-ATAGGTCTGGAGAGTCTTTAAGATTAC-3′ (nt 2521-2495); nested forward 5′-TGTCCTCATTGTAT-TAATGTGGGA-3′ (nt 2127-2150); nested reverse 5′-CAAA-CAGGGAAGATGGGTTT-3′ (nt 2473-2454); amplification region ΔV-II: outer forward 5′-AGGATGTGTATAAGCAA-TTTGTA-3′ (nt 2342-2364); outer reverse 5′-CTTTTCTGA-GGCGTTGTATGC-3′ (nt 2964-2944); nested forward 5′-GTTACTGGGACAGACTTAGAGCTTATA-3′ (nt 2380-2406); nested reverse 5′-ATCTTTTACTGCTTGTGCTTGAA-3′ (nt 2877-2855). For amplification of the region encoding the extreme C-terminal region of the viral structural proteins, the following primers were used (amplification region: VPCext): outer forward 5′-TGGACCAATTGGGGGTATTA-3′ (nt 4315-4334; positions according to genotype 2 strain A6 clone c2, GenBank accession no. AY064475 (17); corresponding sequences of strain LaLi not available); outer reverse 5′-GTTCTCTGCGGGGTATTGG-3′ (nt 4683-4665); nested forward 5′-GAATCCACAGCCTGGAGTGT-3′ (nt 4432-4451); nested reverse 5′-TCTGGGTGGTACAGGAGGAC-3′ (nt 4649-4630).

For DNA sequencing, nested PCR products were purified by QIAquick PCR Purification Kit (Qiagen, Hilden, Germany) and sequencing reactions were carried out using approximately 5-20 ng of the purified PCR product. Amplicons from at least two independent PCR reactions were sequenced in the forward and

reverse directions using the nested primers. Sequencing was performed with the ABI PRISM® BigDye™ Terminator v1.1 Cycle Sequencing Ready Reaction Kit (ABI, Applied Biosystems, Weiterstadt, Germany), unincorporated dye terminators removed using SigmaSpin™ Post-Reaction Clean-Up Columns (Sigma.Aldrich, Steinheim, Germany) and reactions were run on an ABI PRISM® 310 Genetic Analyzer (Applied Biosystems). Sequencing data were manually reviewed. Alignments were generated by ClustalX 1.81 and sequence editing was performed using BioEdit.

Quantitative polymerase chain reaction

For quantitative measurement of genotype 1 DNA contamination, real-time PCR (LightCycler – Parvovirus B19 Quantification Kit, Roche Diagnostics, Mannheim, Germany) was carried out, using a LightCycler instrument (Roche Molecular Biochemicals, Mannheim, Germany), according to the manufacturers instructions. In addition to the Roche B19 DNA standards, the International Standard for B19 DNA (1st World Health Organization International Standard 99/800 for Parvovirus B19 DNA [National Institute of Biological Standard and Control (NIBSC), London, UK]; 5x 10^5 IU per vial) (22) was included in each run. The International Standard was assayed undiluted and in 5 serial tenfold dilutions. The assay amplifies genotype 1 virus. For semi-quantitative measurement of genotype 1 DNA, 10-fold dilutions of the DNA preparations from the factor concentrates, the International Standard for B19 DNA (99/800) and from cloned (almost full-length) B19 DNA were performed. The plasmid pGEM-1/B19 was kindly provided by Dr. Jonathan P. Clewley, Central Public Health Laboratory, London, UK. Nested PCR was performed as mentioned above (19). For semi-quantitative measurement of genotype 2 DNA, a PCR product amplified by the outer primers described by Hokynar et al. (14) was TA cloned into the pCR®4-TOPO® plasmid. Nested PCR with 10-fold dilutions of known amounts of cloned genotype 2 DNA was carried out using the primers described by Hokynar et al. (14) and the amplification protocol given above.

Statistical analysis

The statistical analysis was performed using the χ^2 test.

Results

Contamination with B19 genotype 1 DNA

Genotype 1 DNA was detected by genotype 1-specific PCR in 77/181 (42.5%) lots of coagulation factor concentrates used for therapy in the last three years (Table 1). The percentage of contaminated lots was higher for factor VIII concentrates (47%) than for factor IX (36%) and activated prothrombin complex concentrates (29%). However, the differences were not statistically significant because of the relatively small numbers of factor IX and prothrombin complex concentrate lots. The highest contamination rate was found in product C (74.1%, factor VIII concentrate). Analysis of coagulation factor VIII concentrates used until the beginning of the eighties for therapy showed that the frequency of contamination was significantly higher (17/21 = 81%; $p < 0.01$) than in currently used factor VIII concentrates (Table 2).

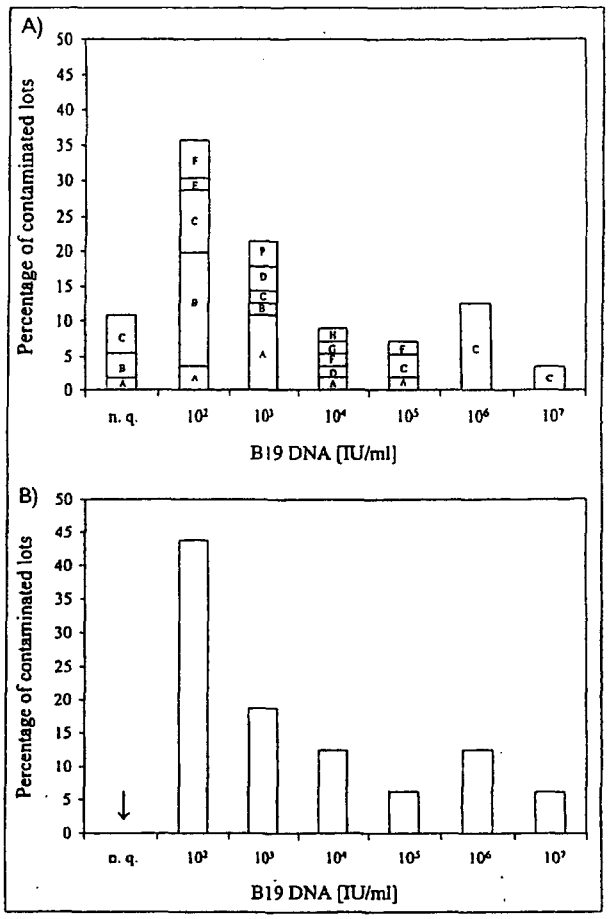

Figure 2: Levels of B19 genotype 1 DNA contamination in coagulation factor VIII concentrates. All PCR-positive lots were quantified by real-time PCR and classified in log10 levels (x-fold value not considered) with the exception of five lots. Figure 2A. Results from 56 currently used lots (four PCR-positive lots not tested). Specific products are indicated by letters in each column. Figure 2B. Results from 16 lots administered until the beginning of the 1980s. Except for one product (product f), all PCR-positive lots were tested. n.q. = not quantifiable.

Quantitative real-time PCR amplifying genotype 1 DNA was performed on 56/60 PCR-positive factor VIII lots currently used and on 16/17 PCR-positive previously used factor VIII lots (Fig. 2). The results showed that contamination ranged from less than 2x 10^2 IU/mL to 3x 10^7 IU/mL. Sixteen percent (9/56) of currently used concentrates proved to be highly contaminat-

Coagulation factor		Genotype 1 (genome equivalents/mL*)	Genotype 2 (genome equivalents/mL)
Currently administered concentrates	C_1	10^{7a}	10^2
	C_2	10^3	10^5
Formerly administered concentrates	b	10^4	10^2
	d	10^5	10^2
	g	10^7	10^5

* 1 genome equivalent is equal to about 0.3 IU of B19
[a] x-fold value not considered

Table 3: Semi-quantitative PCR analysis of parvovirus B19 genotype 1 and 2 DNA in co-contaminated clotting factor concentrates.

ed (10^6 to 10^7 IU/ml). The level of B19 DNA contamination was similar in currently and previously used factor concentrates. In factor IX and prothrombin complex concentrate contaminated lots, viral titres were mostly lower (11/12 and 5/5 lots, respectively, analysed). The maximum viral titre observed in factor IX concentrates was 10^5 IU/ml, and 10^3 IU/ml in prothrombin complex concentrates (data not shown).

Contamination with B19 genotype 2 DNA

Genotype 2 DNA was detected by genotype 2-specific PCR in 2/181 (1.1%) lots of currently administered concentrates (Table 1) and in 3/21 (14%) lots of previously used concentrates (Table 2). Thus, the number of concentrates contaminated with genotype 2 DNA (5/202) was significantly lower than the number of concentrates contaminated with genotype 1 DNA (94/202; $p < 0.001$). All genotype 2 DNA positive lots were co-contaminated with genotype 1 DNA.

To verify the PCR results, DNA sequence analysis was carried out on the five double-PCR positive concentrates using genotype 1 and genotype 2-specific primers in separate sequencing reactions. Because of the low degree of genetic variability of parvovirus B19, sequencing of larger parts of the viral genome was performed. Of the ten isolates, seven isolates (4x genotype 1 and 3x genotype 2 isolates) were sequenced over approximately half of the genome (genotype 1: nt 1901-4708; genotype 2: nt 1901-4830; numbering of nt positions according to genotype 1 prototype strain Au, GenBank accession no. M13178). One genotype 2 DNA isolate was sequenced for 2155 nucleotides (nt 2302-2604, 2973-4830; deletion of six consecutive nucleotides coding for 2 amino acids in the nonstructural 11kD protein). However, due to the considerable quantitative differences in viral contamination between genotype 1 and genotype 2 DNA in two products (see below), successful amplification of larger parts of the low-level contaminating genotype virus was not possible. One genotype 1 isolate could only be sequenced over a region of 677 nucleotides (nt 3361-4037) and one genotype 2 contaminant over 1918 nucleotides (nt 1901-2604, 2973-4008, 4653-4830). Nucleotide sequence comparison was performed with genotype 1 strain Au and genotype 2 strain A6 (accession no. AY064475, AY064476).

Sequence analysis revealed that the double-PCR positive concentrates contained both typical genotype 1 and genotype 2 variants. All genotype 1 and genotype 2 DNA isolates differed from each other. Two of the four genotype 1 isolates that were sequenced over half of the genome revealed ambiguities in 7 and 9 nucleotide positions, respectively. These ambiguities were most probably caused by the presence of several B19 strains within the plasma pool.

In genotype 1 isolates, the percentage of nucleotide positions divergent to genotype 1 prototype strain Au varied from 0.46 to 1.35% (the isolate that could not be sequenced for a larger stretch was not considered). Sequence divergence of the three genotype 2 genomes sequenced for half of the genome to the genotype 2 strain A6 was 1.6, 1.7 and 2.3%, respectively. Genetic difference between the three factor-derived genotype 2 genomes and the genome of genotype 1 prototype strain Au was 8.7 (two isolates) and 9.0% and, therefore, within the range of the divergence between genotype 1 strain Au and genotype 2 strain A6 (9.4%). The nucleotide sequence data from this study have been deposited in the nucleotide database of NCBI (National Center for Biotechnology Information) (GenBank accession numbers AY661660-AY661670. Semi-quantitative PCR analysis of the co-contaminated lots revealed relative differences in viral contamination between the two ge-notypes except for one concentrate. In four concentrates genotype 1 DNA was present in higher concentrations than genotype 2 DNA while in one concentrate (C_2) genotype 2 DNA was present in the higher concentration (Table 3). Because there exists no international standard for genotype 2 DNA, the concentration of genotype 1 DNA is also expressed in genome equivalents to provide better comparability.

Discussion

The purpose of the study presented here was to gain insight into the possible risk of contamination of clotting factor concen-

trates by the recently discovered human parvovirus B19 variant, classified as B19 genotype 2. The results of the study clearly show that genotype 2 DNA is present in coagulation factor concentrates much less frequently than genotype 1 DNA. In currently used coagulation factors the detection rate for genotype 2 was 1.1% whereas the rate for genotype 1 was 42.5% ($p < 0.001$). Although the number of investigated products that have been formerly used was rather small, the study further indicates that the rate of genotype 1 DNA contamination in currently used factor VIII products is significantly lower than in previously used products ($p < 0.01$). Moreover, the fact that genotype 2 DNA has been detected in products used up until the early 1980s indicates that the "new" genotype does not represent a recently emerged virus as might have been assumed due to its recent identification (14, 15, 17).

In literature there is only one report describing prevalence of genotype 2 DNA in human blood. Nguyen et al. (17) tested 62 plasma pools each derived from plasma from 2000 Danish voluntary blood donors. No genotype 2 viraemic pool was detected by PCR. In contrast, screening of the plasma pools for B19 identified 40 pools (65%) containing B19 DNA. Furthermore, among 207 serum samples submitted to the NIH specifically for testing for B19 between 1991 and 2001, only one sample collected from an Italian HIV-positive patient with chronic anaemia tested positive for genotype 2 DNA. Thus, the low detection rate of genotype 2 DNA in clotting factor concentrates observed in the present study is consistent with the low frequency of genotype 2 DNA in blood, and plasma pools.

In contrast to the low detection rate of genotype 2 DNA in blood, we and others have shown that genotype 2 DNA is present in human tissue in a relatively high proportion. Hokynar et al. (14) detected genotype 2 DNA in 9/19 (47%) human skin samples collected from B19 seropositive individuals. Furthermore, we detected genotype 2 DNA in 27/88 (31%) liver specimens collected from randomly selected adults undergoing liver transplantation or liver biopsy or obtained from autopsied individuals (18). Genotype 2 DNA has also been found in 5/83 (6%) livers from patients with fulminant hepatitis or hepatitis-associated aplastic anaemia (23). These findings indicate that genotype 2 is more widespread than might be suspected from the low detection rate in blood or blood-derived coagulation factor concentrates and that genotype 2, like genotype 1, persists in human tissue (24-26).

With regard to the high incidence of viral DNA in tissue and the low detection rate in blood products one can speculate that the characteristics of the viraemic phase of infection might be different between the two genotypes. B19 infection is characterised by a high-level viraemia (up to 10^{12} genome equivalents/ml) during the early stage of infection which is frequently followed by a low-level viraemia, existing for months or even years after acute disease (27). Genotype 2 viraemia, however, might be shorter and viral titres may be mostly lower than in genotype 1 viraemia resulting in a low occurrence rate in blood products.

Alternatively, it is conceivable that divergence between the structural proteins of the two genotypes mediates an altered sensitivity to the virus removal/inactivation procedures used during the manufacturing of coagulation factor concentrates. However, this hypothesis seems unlikely because the sequence divergence is relatively low, i.e. 1.4 to <2% at the amino acid sequence level for the major viral capsid protein (VP2) which accounts for about 95% of the viral capsid, and 2.2 to 3.3% for the minor viral capsid protein (VP1) present in the virion. This calculation is based on the genotype 2 sequences presented herein, together with those available from GenBank. However, to unambiguously rule out the possibility that the small differences in capsid composition between the two genotypes would mediate a different sensitivity to physical and chemical procedures, specific culture studies with genotype 2 would be necessary. Although there is some experience from work with genotype 1 (28-32), successful propagation of genotype 2 in cell culture as a prerequisite for such investigations has not been reported up to now.

Genotype 1 viral DNA is frequently present in currently administered factor concentrates. The occurrence of blood donations contaminated with genotype 1 DNA has been estimated to be between 1: 5950 to about 1: 30000 (33, 34), increasing to as high as 1: 260 during epidemic periods (35). The high levels of viraemia in acutely infected individuals combined with the resistance of the virus to inactivation procedures, means that there is a high probability of lot contamination. Factor VIII products were found to contain the highest degree of genotype 1 contamination (10^6 and 10^7 IU/ml). However, these maximum levels were present only in the product from one manufacturer (product C). Over the last few years, nucleic acid testing (NAT) of plasma pools for B19 DNA has been increasingly implemented. In 2002 it was stated by a plasma protein consortium (PPTA) that NAT for B19 DNA has now became universally effective and manufacturing pools will not exceed levels of 10^5 IU DNA/ml. Furthermore, although a recommendation was presented suggesting a standardised B19 NAT schedule to ensure that the proposed limit of the FDA for manufacturing pools (<10^4 IU/ml) can be achieved, the application of NAT assays to plasma pools, destined for production of coagulation factor concentrates, remains a voluntary procedure. However, the fact that the overall frequency of viral contamination in currently administered products is significantly lower than in formerly used ones might be interpreted as a positive effect of the present procedures, including NAT, to reduce the risk of contamination of clotting factor products.

The results presented here indicate that genotype 2 is not a frequent contaminant (2.5%) in coagulation factor concentrates. Nevertheless, to further improve the viral safety of blood products it seems reasonable to reflect on the need for implementa-

tion of nucleic acid testing for genotype 2. Since parvovirus B19 has been recognized as a major contaminant of blood products, plasma pool testing by NAT for genotype 1 is now widely applied. However, it can be assumed that, similar to our standard PCR for B19, many primers currently used for B19 PCR do not detect genotype 2 DNA because of insufficient complementarity. Thus, for detection of genotype 2, and possibly genotype 3 DNA, alternative primers such as consensus primers or degenerate primers should be used if separate amplification is to be avoided. Since there is evidence that genotype 2 causes the same spectrum of diseases as genotype 1, development of a PCR system able to detect DNA from both genotypes of the human parvovirus B19 would give an added level of safety to blood products.

Acknowledgement

The authors would like to express their gratitude to Ulrike Reber for excellent technical assistance.

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別紙様式第２－１

医薬品　研究報告 調査報告書

識別番号・報告回数			報告日	第一報入手日	新医薬品等の区分	総合機構処理欄
一般的名称	－		研究報告の公表状況	AABB Weekly Report2006:12(32)10.	公表国 米国	
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
自動車事故後に輸血を受けた患者１例がその後マラリアと診断され、疑わしい血液を認識していたにもかかわらず、流通を防げなかったとして韓国赤十字は批判を受けている。 不適切な血液スクリーニング手順にも関わらず韓国赤十字は、プライバシーに関する法律をもとにマラリア感染率が高い地域に関する情報を韓国 CDC (Korea Center for Disease Control and Prevention) が提供しなかったことが原因であるとしている。	

報告企業の意見	今後の対応
韓国において、輸血によりマラリアが感染したとの報告である。 血漿分画製剤によりマラリアが感染したとの報告はない。 なお、日本赤十字社では、輸血によるマラリア感染を防ぐため、WHOの指定しているマラリア流行地域に旅行した人については１年間、居住した人については３年間、献血を禁止している。	今後ともマラリアに関する安全性情報等に留意していく。

An article in the *Japan Times* further noted that the government was found liable for failing to prevent the use of these products. In handing down his ruling, Judge Keiji Suda said that the state and the company were negligent because they allowed the use of unheated blood products despite the knowledge of the dangers associated with their use.

The recent distribution of blood containing malaria has prompted public concern regarding the Korean National Red Cross and its management of blood and blood products. According to a Sept. 8 *Korea Times* article, a patient who received a transfusion after a car accident and was subsequently diagnosed with malaria has criticized the organization for failing to prevent the distribution of suspect blood. Some allege that the Korean National Red Cross was made aware of the situation but did not take any action, resulting in continued circulation of unsuitable blood products. One patient has reportedly already died after contracting malaria via transfusion.

According to the article, despite inadequate blood screening procedures, the Korean National Red Cross attributes the problem to a privacy law that prevents it from viewing data from the Korea Center for Disease Control and Prevention, which holds information on regions with a high rate of malaria infection.

Industry

Abbott Laboratories will soon deliver 20 of its PRISM system fully automated blood screening instruments to ARC U.S. National Testing Laboratories. In addition to the PRISM systems, the company will also provide the ARC laboratories with the hepatitis B core antibody and surface antigen assays. According to a Sept. 6 press release posted on PR Newswire, the contract for the equipment and assays begins immediately and is expected to run through 2011. "This agreement underscores our longstanding commitment to working with the American Red Cross to help ensure the safety of the nation's blood supply," said Jeff Binder, senior vice president of diagnostic operations at Abbott.

People

Kathy Connolly, chair of the AABB Donor Recruitment and Public Relations Committee, was recently recognized for her commitment to the blood community. Connolly, who began her career in blood banking more than 30 years ago, works as the director of public relations for the Rhode Island Blood Center and was presented with the Bank of America's 2006 Neighborhood Champions in acknowledgment of her dedication to health care. In 2003, Connolly was awarded the AABB Chapman-Franzmeier Memorial Award for her local and national efforts to recruit blood donors.

別紙様式第2-1

No. 3

医薬品　研究報告　調査報告書

識別番号・報告回数			報告日	第一報入手日	新医薬品等の区分	機構処理欄
				2006. 6. 26	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	CDC. 2006 Jun 16; Available from: URL: http://www.cdc.gov/travel/other/2006/malaria_bahamas.htm	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）			

研究報告の概要

○マラリア発生警告（バハマ、グレートエグズーマ島）
2006年6月中旬、米国疾病対策予防センター（CDC）は、バハマのグレートエグズーマ島におけるマラリア発生確認の報告を受領した。当該地域では、通常はマラリアの伝播は起こらず、これまで抗マラリア薬の使用は勧告されていなかった。
6月29日時点で合計18例が発生した。このうち4例は旅行者で、出身国は米国2例、カナダ1例、及びドイツ1例であり、滞在期間は4月後半から5月末の間であった。
これらの症例は、いずれもPlasmodium falciparum による感染症（熱帯熱マラリア）であることが確認されている。感染したバハマ住民の一部は、流行地域であるハイチを最近旅行した可能性がある。6月19日以降は新たな感染例は確認されていない。
CDCは米国からグレートエグズーマ島への旅行者に対して、十分な予防効果のある高用量のクロロキンの使用を勧告している。この勧告は一時的なものであり、バハマ諸島の他の島は該当しない。
抗マラリア薬の予防効果は100%ではないため、当地への旅行者は蚊に刺されないよう予防策を講ずること。当地では、蚊媒介性の他の疾患が起こっているため、バハマ諸島の他の島に向かう旅行者も同様に予防策を取ること。
外出時、特に日没から日出の間には、露出した皮膚の表面に防虫剤を塗布すること。30%～50% DEET（N, N-diethyl-m-toluamide）を含有する防虫剤が推奨される。低濃度の場合は防虫効果が短時間となるため、より頻繁に使用する必要がある。
熱帯熱マラリアは迅速に治療しないと重症となり生命を脅かす疾患である。当地に旅行して、発熱やインフルエンザ様症状を発症した場合、直ちに専門医の治療を受けること。このとき、医療機関でマラリアの発生地域へ旅行した旨を伝えること。

報告企業の意見	今後の対応
米国疾病対策予防センターは、バハマのグレートエグズーマ島におけるマラリア発生を確認し、旅行者に対して抗マラリア薬の使用を勧告したとの報告である。	日本赤十字社は、8月1日以降、バハマに滞在した場合は帰国（入国）から1年間献血延期としている（帰国（入国）後にマラリアを思わせる症状があった場合は、マラリア感染が否定されるまで）。また、今後も引き続き、マラリア感染に関する新たな知見及び情報の収集に努める。

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」

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

Travelers' Health

Outbreak Notice

Update: Malaria, Great Exuma, Bahamas

This information is current as of today, July 4, 2006, 04:39:43 PM

Updated: June 30, 2006
Released: June 16, 2006

In mid-June 2006, the Centers for Disease Control and Prevention (CDC) received official reports of confirmed malaria cases in Great Exuma, Bahamas, an area where malaria transmission does not normally occur and for which antimalarial drugs have not previously been recommended. As of June 29, there have been a total of 18 cases of which 4 were travelers. Of the 4 travel-associated cases, 2 were from the U.S., 1 from Canada, and 1 from Germany. All had traveled to Great Exuma for varying periods between late April and the end of May.

All these confirmed infections were caused by *Plasmodium falciparum*. Most of the patients reported no recent travel to malaria-endemic areas, but some of the Bahamas residents diagnosed with malaria may have recently traveled from Haiti, where *P. falciparum* is endemic. No additional cases of malaria have been identified since June 19.

Malaria is not considered endemic on the islands of the Bahamas. The Ministry of Health in the Bahamas has responded with heightened surveillance for and treatment of malaria cases, mosquito control measures, and education of the local population. The Caribbean Epidemiology Center and the Pan American Health Organization/World Health Organization are assisting the Ministry of Health with these response measures.

Antimalarial Medication

At this time, CDC is recommending that U.S. based travelers take preventive doses of chloroquine before, during, and after they travel to Great Exuma. This recommendation is expected to be temporary and does not apply to other islands of the Bahamas. Chloroquine has a long history of use and safety and is well tolerated by most people, including children. People with an allergy to chloroquine should discuss an alternative antimalarial drug with their health-care provider. To learn more about chloroquine, including dosing information, see Information for the Public: Prescription Drugs for Malaria.

Other Prevention Measures

Because chloroquine and other antimalarial drugs are not 100% protective, travelers to Great Exuma should take precautions to protect against mosquito bites. These prevention measures should be taken by travelers to other islands in the Bahamas as well because other mosquito transmitted infections occur there.

- Use insect repellent on exposed skin surfaces when outdoors, particularly from dusk to dawn. Repellents containing 30% – 50% DEET (N, N-diethyl-m-toluamide) are recommended. Lower concentrations of DEET offer shorter-term protection, requiring more frequent reapplication.
- To learn more about preventing mosquito bites and the appropriate use of insect repellents, visit Protection Against Mosquito and Other Arthropods in *Health Information for International Travel* and What You Need to Know about Mosquito Repellent.

Malaria caused by *P. falciparum* may rapidly result in a severe, life-threatening illness if not promptly treated. If you have traveled to Great Exuma and you become ill with fever and other flu-like

symptoms, you should immediately seek professional medical care; inform your health-care provider that you have visited an area currently experiencing a malaria outbreak.

Visit the CDC's Travelers' Health website for Health information for Travelers to Countries in the Caribbean.

Additional information about malaria can be found at the CDC Malaria homepage.

Health-care providers needing assistance with diagnosis or management of suspected cases of malaria should call the CDC Malaria Hotline: 770-488-7788 (M-F, 8 am-4:30 pm, Eastern Time). For consultation after hours, call 770-488-7100 and ask to speak with a CDC Malaria Branch clinician.

Date: June 30, 2006
Content Source: National Center for Infectious Diseases, Division of Global Migration and Quarantine

Centers for Disease Control and Prevention,1600 Clifton Rd, Atlanta, GA 30333, U.S.A
Tel: (404) 639-3311 / Public Inquiries: (404) 639-3534 / (800) 311-3435