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一般的名称	乾燥濃縮人アンチトロンビンⅢ		研究報告の公表状況	Transfusion;45,1003-1010, 2005-07-27 Parvovirus B19 transmission by a high-purity factor VIII concentrate	公表国 米国	
販売名（企業名）	アンスロビンP-ベーリング（ZLBベーリング株式会社）					

研究報告の概要

問題点（ヒトパルボウイルスB19のNATスクリーニング検査未実施による感染）

47歳の血友病A患者が結腸内視術施行前に予防のため、S/D処理血液凝固第VIII因子製剤の同一ロット3Vと他のロット1Vで、総量約3800IUの投与を受けた。

患者は投与10分前に採血していた。その後2週間、インフルエンザ様の微熱、疲労感、断続的な腹痛と重度の関節痛があった。術後4週目に検査したところ、抗B19 IgMとIgGが陽性であることが判った。

血液凝固第VIII因子製剤投与前の検体を測定すると、抗B19 IgMとIgG、B19DNAが陰性、投与後の検体はすべて陽性であった。患者は結腸内視術前の少なくとも12年間は、血液凝固第VIII因子製剤や血液製剤の投与は受けていなかった。患者はELISA法で血清学的検査をしたが、HCV陽性、HAV、HBV、HIVは陰性であった。

その他3人の患者が同じ病院で、感染疑いのあるロットの製剤の投与を受けていた。その患者は頻繁に血液凝固第VIII因子製剤の投与を受けているが、B19に関連した症状は発現していない。

感染疑いのあるロットの製剤と感染の因果関係を決定するため、患者の投与後の検体、感染疑いのあるロットの製剤とそのロットのプール血漿の遺伝子配列を調べたところ、極めて類似していたが、WHOなどの標準B19の塩基配列とは異なっていた。

感染疑いのあるロットの製剤のB19DNAは$1.3x10^3$ geq/mLあり、米国でB19のNATのスクリーニング検査を実施していない血漿から製造された製剤のB19DNAレベルと同じレベルであった。

血漿分画製剤による伝播の潜在的危険性を低減するため、製造業者はハイタイターのミニプールNATスクリーニングを導入している。B19のNATスクリーニングを実施していたならば、今回報告した感染は起こらなかったかもしれない。B19 DNAのレベルの高いドナーが同定できれば、感染疑いのあるロットのミニプールは存在していなかった。

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
ZLBベーリングでは、B19のNAT検査を実施しており、ウイルス値が高値なものを除外しているので、感染のリスクは極めて低いと考えられる。	今後とも感染症に関する情報収集に努める所存である。

Parvovirus B19 transmission by a high-purity factor VIII concentrate

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BACKGROUND: Parvovirus B19 (B19) is known to cause a variety of human diseases in susceptible individuals by close contact via the respiratory route or by transfusion of contaminated blood or blood products. In this study, whether a case of B19 transmission was causally related to the infusion of implicated lots of a solvent/detergent (S/D)-treated, immunoaffinity-purified factor VIII concentrate (antihemophilic factor [human] [AHF]) was investigated.

STUDY DESIGN AND METHODS: Anti-B19 (both immunoglobulin M [IgM] and immunoglobulin G [IgG]) and B19 DNA (by a nucleic acid testing [NAT] procedure) were assayed in two implicated product lots, a plasma pool, and a recipient's serum sample. Analysis of the partial B19 sequences obtained from sequencing clones or direct sequencing of the samples was performed.

RESULTS: Only one of the two implicated lots was B19 DNA–positive. It contained 1.3×10^3 genome equivalents (geq or international units [IU]) per mL. The negative lot was derived from plasma screened for B19 DNA by NAT in a minipool format to exclude high-titer donations, whereas the positive lot was mostly from unscreened plasma. This high-purity AHF product had no detectable anti-B19 IgG. A 4-week postinfusion serum sample from a recipient, who received both lots and became ill, was positive for the presence of B19 antibodies (both IgM and IgG) as well as B19 DNA. The B19 sequences from the positive lot, its plasma pool, and the recipient's serum sample were closely related.

CONCLUSION: These findings and the recipient's clinical history support a causal relationship between the implicated AHF product and B19 infection in this recipient. The seronegative patient became infected after receiving 2×10^4 IU (or geq) of B19 DNA, which was present in this S/D-treated, high-purity AHF product.

Parvovirus B19 (B19) is a small, nonenveloped, single-stranded DNA virus that is known to resist viral inactivation procedures commonly used in manufacturing plasma-derived products. The virus is widespread, and manifestations of infection vary depending on the immunologic and hematologic status of the host (there are numerous reviews; see Young and Brown[1] for a recent review). B19 infection in immunocompetent persons is often asymptomatic or benign, or results in mild illness, including erythema infectiosum (fifth disease) in children and arthropathy in adults. B19, however, can cause more severe diseases in vulnerable individuals, such as transient aplastic crisis in persons with hematologic disorders, hydrops fetalis and subsequent congenital anemia or fetal death in pregnant women, and pure red cell aplasia and chronic anemia in those who are immune compromised.[1]

Diagnosis of B19 infection is based on detection of specific immunoglobulin M (IgM) and immunoglobulin M (IgG) antibodies by enzyme-linked immunosorbent assays (ELISA) or of viral DNA by nucleic acid testing (NAT) procedures. Serologic studies have shown that at least 50 percent of adults have circulating B19 IgG, which is evidence of past infection.[2,3] The prevalence of B19 viremia in blood and plasma donors has been reported to

ABBREVIATIONS: AHF = antihemophilic factor; Human B19 = parvovirus B19; HTC = hemophilia treatment center.

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range from 0.003 to 0.6 percent, depending on whether the testing is performed at the time of an epidemic and the sensitivity of NAT methods.[4-6] Although B19 is normally transmitted via the respiratory route or transplacentally from mother to fetus, parenteral transmission can occur through the infusion of contaminated blood products. These latter transmissions are mainly due to the extremely high viremic levels in plasma, for example, 10^{13} genome equivalents (geq) of B19 DNA per mL, found at an early phase of the infection in acutely infected but asymptomatic donors.[7] Thus, the risk of B19 transmission via single-donor blood components is rare but is substantially greater via products from pooled plasma.[8]

B19 DNA has been detected in high proportions and high levels in plasma pools and their resulting plasma derivatives, especially the coagulation products.[9-11] Consistent with these findings, almost all persons with hemophilia in cross-sectional studies from 1982 to 1997 were found to be anti-B19 IgG–positive, in contrast to approximately 50 percent of the untransfused, untreated control population.[12] Reports of transmissions attributed to infused factor VIII (FVIII) concentrates (antihemophilic factor [human] [AHF]), subjected to solvent/detergent (S/D) or heat treatment or both, have been numerous.[12-17]

In most transmission cases, however, the causality assessment[18] between the product and the recipient's infection was not well established. The level of B19 DNA in the product that resulted in infection has rarely been determined, and the minimum viral exposure needed to produce an infection is not clearly defined. In this study, we investigated a case report and established a causal relationship between an implicated AHF product and a recipient. Infection occurred when the seronegative recipient received 2×10^4 geq (equivalent to 2×10^4 international units [IU] based upon our NAT method[19]) of B19 DNA, in a S/D-treated, high-purity AHF product that contained no detectable anti-B19 IgG. Two product lots were initially implicated. One lot, which was negative for B19 DNA, was derived solely from plasma screened for B19 DNA by NAT in a minipool format to exclude high-titer donations.

CASE REPORT

The Food and Drug Administration (FDA) received the following MedWatch report (an adverse event report) from a hemophilia treatment center (HTC) in 2001. A 47-year-old man with mild hemophilia A received a total of approximately 3800 IU of FVIII clotting activity in the form of three vials of one lot of AHF and one vial of another lot, as prophylaxis before undergoing colonoscopy. Both lots were the same AHF product. The patient had a blood specimen drawn 10 minutes before the product infusion for participation in an HTC-based blood safety monitoring program sponsored by the Centers for Disease Control and Prevention (CDC).[20] Beginning 1 week after the colonoscopy, he experienced 2 weeks of symptoms, including flu-like low-grade fever, extreme fatigue, intermittent abdominal pain, and significant joint pain. He visited his primary care physician 4 weeks after the procedure and a blood specimen was taken. It was found to be positive for the presence of anti-B19 IgM and IgG. The HTC notified the manufacturer and CDC, and the pre- and postinfusion specimens were tested at the CDC. The CDC found that the preinfusion sample was negative for the presence of anti-B19 IgM and IgG and for B19 DNA, whereas the postinfusion sample was positive for all three markers. Before the colonoscopy, the patient had not used any AHF or blood products for at least 12 years. He was positive serologically for the presence of hepatitis C virus but negative for the presence of hepatitis A virus, hepatitis B virus, or human immunodeficiency virus, all of which were performed by ELISA tests. He had no children and denied any association with children during the weeks before and following the colonoscopy.

The CDC informed the FDA of the incident and both agencies jointly investigated the case. Three other patients also received the implicated lots from the same treatment center. All three of these patients were retrospectively found to be frequent users of AHF products and did not experience any discomfort or B19-associated symptoms during the same time period.

MATERIALS AND METHODS

Patient specimens, AHF, and plasma pool samples

Serum specimens were collected from the patient before and 4 weeks after the infusion. Lot-release samples of the two implicated AHF lots (designated A and B) submitted by the manufacturer to the FDA were available. Several plasma pools used as starting material for manufacturing lot A were kindly provided by the manufacturer.

Detection and quantitation of B19 DNA by polymerase chain reaction

One vial of each implicated AHF lot was reconstituted with 5 mL of diluent, that is, half of the volume specified by the manufacturer, and 0.2-mL aliquots were used for DNA extraction. For serum specimens or plasma pools, 0.1-mL aliquots were used. For a plasma pool having a high titer of DNA, a 10^3-fold diluted sample was used. DNA was extracted by use of an isolation kit and procedures (NucliSens, Organon Teknika, Durham, NC), and the DNA from each sample was recovered with 100 μL of elution buffer. The following in-house nested B19 NAT procedure was used. A 25-μL aliquot of undiluted or $10^{0.5}$-fold serially diluted DNA extract was added to a 25-μL master mix so that the final reaction mixture for the first-round amplification contained 10 mmol per L Tris-HCl, pH 8.3,

50 mmol per L KCl, 1.5 mmol per L $MgCl_2$, 0.001 percent (wt/vol) gelatin, 1.25 units AmpliTaq DNA polymerase (Applied Biosystems, Foster City, CA), 100 µmol per L of each dNTP, and 0.2 µmol per L of each of the first-set primers. One microliter of the first amplification product was transferred into a second 50-µL amplification mixture containing the same constituents as the first except that 0.2 µmol per L of each of the second-set primers was present. Nested primer sequences derived from the VP1/VP2 region of the B19-Au genome[21] were used. The first-set primers were 5′-CTTTAGGTATAGCCAACTGG-3′ (nucleotides 2905-2924) and 5′-CCTTATAATGGTGC TCTGGG-3′ (nucleotides 3290-3271), whereas the second-set primers were 5′-CATTGGACTGTAGCAGATGA-3′ (nucleotides 2951-2970) and 5′-GCTTTTGACAGAATTA CTGC-3′ (nucleotides 3193-3174). Amplification was performed in a thermocycler (Model 9600, Perkin Elmer, Foster City, CA) with the following settings: for the first-round amplification, an initial heating at 94°C for 3 minutes was followed by 30 cycles of 94°C for 30 seconds, 55°C for 30 seconds, and 72°C for 90 seconds; for the second amplification, 30 cycles of 94°C for 15 seconds, 55°C for 30 seconds, and 72°C for 50 seconds were performed. A final amplification product of 243 bp was then analyzed by electrophoresis on 1.8 percent agarose gel and visualized by ethidium bromide staining.

For sample extraction and B19 NAT, both the WHO International Standard (NIBSC 99/800, 10^6 IU of B19 DNA/ mL when reconstituted) and the CBER standard for B19 DNA (10^6 IU/mL) were used as controls.[19] Both were diluted 10^3-fold before use. The amount of B19 DNA expressed as geq was determined by limiting dilution analysis. For 0.1 mL of serum or plasma, the sensitivity of the NAT was 40 geq per mL; for 0.2 mL of AHF, it was 20 geq per mL. Both standards were found to contain 10^6 geq per mL by our NAT procedure and hence the conversion ratio from IU to geq is 1:1, rather than 1:0.6 to 1:0.8 obtained in a collaborative study.[19]

Cloning, sequencing, and phylogenetic analysis

The extracted DNA samples were amplified with a seminested B19 NAT procedure for DNA sequencing. Both antisense primers, other NAT constituents, and conditions were similar to those described above for the nested B19 NAT procedure except that a new sense primer derived from the C-terminal NS1 region, that is, 5′-GTGCTTACCT GTCTGGATTG (nucleotides 2408-2427), was used for both rounds of amplification so that the C-terminal NS1 region and the unique VP1 region would be included in the amplified product. The final amplified product, 786 bp, was purified by a polymerase chain reaction (PCR) purification kit (QIAQuick, Qiagen, Hilden, Germany), introduced into the pCR2.1-TOPO vector (Invitrogen, Carlsbad, CA), and subsequently was used to transform *Escherichia coli*. For each of the samples, as well as the two NAT controls (WHO and CBER B19 standards), three to six clones were grown and purified by use of a spin miniprep (QIAPrep, Qiagen).

The inserts were sequenced on a sequencer (Model 310, Applied Biosystems) with a cycle sequencing kit containing a high-fidelity DNA polymerase (Big Dye Terminator, Applied Biosystems) with 0.5 µg of plasmid DNA and a universal M13 reverse primer according to the manufacturer's specified procedure. Cloning was not performed on the plasma pool sample that contained high levels of B19 DNA. Instead, the amplified products derived from the pool sample, as well as from both B19 NAT standards, were sequenced directly. The sequencing primers were the same as those used for the seminested NAT procedure. Sequence alignments of the N-terminal unique VP1 region corresponded to nucleotides 2444 through 2666 (nucleotide numbering based on the published Au sequence[21]), and phylogenetic analysis was performed by with a computer program (DNASTAR, DNASTAR, Inc., Madison, WI). Trees were generated by use of a neighbor-joining algorithm in comparison to 12 published B19 sequences adopted from GenBank.

Anti-B19 detection

Anti-B19 IgM and IgG antibodies were detected by use of B19 IgM and IgG enzyme immunoassay kits (Biotrin International Ltd, Dublin, Ireland) according to the manufacturer's protocols. For serum or plasma specimens, 10-µL aliquots were used as instructed, whereas for AHF, a 100-µL aliquot was used.

RESULTS

The 4-week postinfusion serum sample from the patient was found to be positive for the presence of anti-B19 (both IgM and IgG) and B19 DNA, whereas the serum sample collected before the infusion of the implicated AHF lots was negative for both (Table 1). The viremic level in the 4-week specimen was 10^3 geq per mL (equivalent to 10^3 IU/mL) as determined by limiting dilution analysis with our nested NAT method. Only AHF lot A had detectable B19 DNA, that is, 1.3×10^3 geq per mL (or 6.5×10^3 IU/vial of AHF). Anti-B19 IgG was not detected in the product. Hence, the patient received a total of 2×10^4 geq (or IU) of B19 DNA with no anti-B19 IgG associated with the AHF product. Several plasma pools from which lot A was derived were subsequently tested and all but one contained less than 10^4 geq per mL B19 DNA (data not shown). That plasma pool contained 10^7 geq per mL of B19 DNA and was positive for the presence of anti-B19 IgG, as expected for any given large plasma pool.

To investigate the case further, DNA sequencing of the C-terminal NS-1 region and the unique VP1 region was

performed. The unique VP1 sequences obtained from clones derived from the recipient and the implicated AHF product were compared to clones derived from the WHO and CBER B19 DNA standards, direct sequences obtained from the high-titer plasma pool for lot A, the two B19 DNA

TABLE 1. Anti-B19 and B19 DNA in patient's serum specimens, implicated AHF lots, and a sample of a plasma pool used in manufacturing for lot A

Sample	B19 antibodies IgM	B19 antibodies IgG	B19 DNA (geq/mL)*
Patient			
Preinfusion serum sample	Negative	Negative	Negative
4-week serum sample	Positive	Positive	1000
Implicated AHF			
Lot A	ND†	Negative	1,300
Lot B	ND	ND	Negative
Plasma pool for Lot A	ND	Positive	10^7

* Calculated from geometric mean values of two determinations for the postinfusion sample, four determinations for the AHF sample, and two for the plasma pools. The conversion ratio from geq to IU of B19 DNA by the NAT method is 1:1 (see Materials and methods).
† ND = not determined.

standards, and 12 published B19 sequences including strains Au[21] and Wi[22] in GenBank. As summarized in Table 2, two unique nucleotide substitutions were identified at positions 88 and 135, equivalent to 2531 (GTT to CTT) and 2578 (TAT to TAC) of B19 Au strain, in all five clones (Pat-1-5) from the patient, in all four clones (VIII-1-4) from the implicated lot A, and in a direct sequence obtained from the high-titer plasma pool (Plasma-P). In contrast, both substitutions were not found in any of the published isolates or in any of the six clones (WHO-1-6) or in the direct sequence derived from the WHO standard (WHO-P). Although one of three clones derived from the CBER B19 standard, that is, CBER-1, had both substitutions, it also had additional sequence variations elsewhere within the region. Identical sequences were obtained from all five (Pat-1-5) clones from the patient, three (VIII-1, -2, and -4) clones from implicated product lot A, and the direct sequence from a plasma pool for lot A, that is, Plasma-P. VIII-3 had two additional substitutions in the VP1 unique region. Variability of the clone sequences from both WHO and CBER standards was also seen; however, it was rarely detected by direct sequencing (Table 2).

To demonstrate a causal relationship between the implicated product and the patient's infection, phylogenetic analysis of the sequences was performed. Nucleotide sequences from both the patient's postinfusion serum sample and AHF lot A and the predominant sequence of the plasma pool for lot A were highly similar (Fig. 1). In contrast, these sequences were not closely related to those derived from either the WHO and the CBER standards or other known B19 isolates.

DISCUSSION

Adverse event reports are routinely received by the FDA (often through the MedWatch system), and every effort is made to follow-up reports of disease transmission by FDA-regulated products. Unfortunately, in the case of putative infectivity, preinfusion samples of the recipients' serum samples are rarely available; hence causality is difficult to assess. In the case investigated in this study, a preinfusion specimen obtained immediately before administration of the implicated product was uniquely available for analysis.

The recipient's seroconversion to B19-specific IgM and IgG antibodies, and the appearance of B19 DNA, occurred in the same time as symptoms consistent with acute B19 infection.[1] The low viremic level, 10^3 geq per mL, found in the patient's 4-week postinfusion specimen was consistent with levels found after seroconversion.[7] Although serum samples of other patients who received the implicated lots at the same hospital were not available, it is reasonable to assume that they had seroconverted at some time in the past because they were all frequent users of AHF products. Evidently, as a result of the protection afforded by their circulating antibodies, they did not experience any B19-associated symptoms.

B19 DNA has been known to be prevalent in plasma-derived AHF products with documented levels as high as 10^7 geq per mL of reconstituted product.[9,11] In our study, the level of B19 DNA found in the implicated AHF product (lot A) was 1.3×10^3 geq per mL, which is near the mean level of B19 DNA historically found in US-licensed AHF products derived from plasma unscreened by B19 NAT.[23] As expected, the level of B19 DNA found in the plasma pool from which lot A was derived was much higher, that is, 10^7 geq per mL, although levels of at least 10^8 geq per mL have been reported.[11]

B19 is a small nonenveloped virus known to withstand the commonly used virucidal method for plasma derivatives, such as S/D and heat treatments, although recent findings[24,25] suggest that B19 could be susceptible to inactivation when heated in certain liquid media. Hence, the clearance of B19 for plasma derivatives relies mainly on removal, rather than inactivation, steps, such as chromatography or nanofiltration,[26] the latter being effective only when small pore-size membranes are used. Although the purification procedures for the AHF-implicated product include an immunoaffinity-chromatography step, which has been validated to remove effectively a model virus for B19, the viral load in the manufacturing pool obviously was too high to clear the virus from the final product. Additionally, we found that B19 IgG antibodies, which are considered to be neutralizing antibodies and appear to confer lasting protection,[1] were present in the large plasma pool but not detected in the final product.

TABLE 2. B19 nucleotide sequences of the VP1 unique region among various isolates

Nucleotide:*	9	10	12	34	43	51	53	54	61	71	72	73	76	84	87	88	89	90	96	97	105	106	107	108	129	132	135	138	144	150	151	171	173	174	175	179	180	181	184	203	208	214	217	218
Consensus	A	G	A	G	T	A	C	T	C	T	G	G	T	A	G	G	T	T	A	A	A	G	A	G	T	T	T	T	T	T	A	C	C	T	C	T	T	G	T	T	C	A	T	C
B19-Au		A																																										
B19-Wi														G																	C							A						
Pat-1																C										C																		
Pat-2																C										C																		
Pat-3																C										C																		
Pat-4																C										C																		
Pat-5																C										C																		
VIII-1																C										C																		
VIII-2																C										C																		
VIII-3						G							C			C										C																		
VIII-4																C										C																		
Plasma-P																C										C																		
WHO-1																					T		C																					
WHO-2																	C	C																											
WHO-3																					C		G																					
WHO-4																		C			T	T	G												C									
WHO-5																																												
WHO-6										C										T													A											
WHO-P																																												
CBER-1																C					C		C				C										A						
CBER-2										A											T		C					C															
CBER-3																					C		C																					
CBER-P																							C					C															
AB126262†																																												
AB126271†																																												
AJ717293†		A				G	A	C	A		A				A				G								C					T		C					G	T	G	A	
AY582161†																																			T				C					T
AY582163†				A											A														C	C														
AY582164†					C										A																													
AY647977†	T		C	A	C	G	A	C	A	C	A	C			A		C		T		T			A	A	C		C				T	A	C	T			C	G	T				
AY661660†								C	G		A																				C												
AY661663†						G	A	C	A		A				A				G								C					T		C					G	T	G			
NC000883†																										C																		

* Nucleotide numbering of the consensus sequence is based on the published B19 Au strain, between nucleotide 2444 and nucleotide 2666.
† GenBank accession numbers of the published B19 sequences.

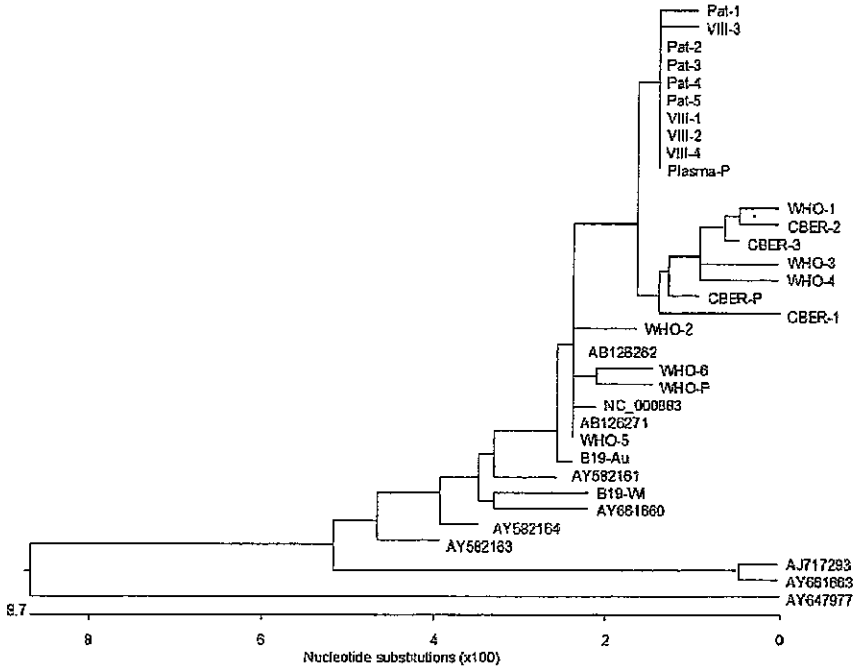

Fig. 1. Estimated neighbor-joining tree obtained from B19 sequences (223 nucleotides) of the VP1 unique region obtained from the patient's 4-week postinfusion serum sample (Pat clones 1-5), from lot A of AHF (VIII clones 1-4), from the CBER (CBER clones 1-3), and WHO B19 (WHO clones 1-6) standards and sequences determined directly from PCR-amplified products derived from either the plasma pool containing 10^7 geq per mL B19 DNA for lot A (Plasma-P) or the two corresponding B19 standards (WHO-P and CBER-P). Included for analysis are 12 published B19 sequences including strains Au and Wi in GenBank. Relative molecular distances are indicated as the number of nucleotide substitutions per hundred nucleotides.

Detection of B19 DNA in both the product and the recipient does not necessarily equate with causality. To further establish a causal relationship,[18] we explored the genetic evidence of B19 sequence similarity among the recipient's serum sample, the infused AHF product, and a plasma pool for the product. In general, the genetic diversity among B19 isolates is very low, with less than 1 to 2 percent nucleotide divergence in the whole genome.[17,27] To confirm two B19-transmission cases by their corresponding coagulation products, Blümel and coworkers[17] sequenced about half of the B19 genome (approx. 2700 nucleotides) consisting of the C-terminal NS-1 region, the VP1-unique region, and the VP2 region. These authors employed direct sequencing of PCR-amplified products, which reflects only the predominant sequence, but not minor variant sequences that may be present in the implicated products. We chose to sequence mainly the unique VP1 region, which appeared to exhibit the most variation in sequences at both the DNA and the protein levels.[17,28] Because there might be many contaminated plasma donations, resulting in a mixture of B19 sequences in the final product, we performed sequence cloning of PCR-amplified DNA from the patient's serum sample and the implicated product to reveal individual sequences present in the original donations.

The sequences of all five clones from the recipient's serum sample were not only identical to three of the four clones from the implicated product, but also to the predominant sequence from the product's plasma pool. Two unique nucleotide substitutions were observed in all sequences from the patient, the product, and the plasma pool. Phylogenetic analysis revealed a close relationship among sequences from all three sources, whereas the sequences determined for the WHO and CBER B19 standards and the published Au, Wi, and 10 other B19 isolates were distinct. In addition, all sequences mentioned in this study were confirmed as genotype 1, distinctively different from strain Lali (genotype 2) and strain V9 (genotype 3) sequences (data not shown).[27]

As a result of the B19 transmission associated with pooled plasma, S/D treated[29-32] in a postmarket surveillance study that correlated product infectivity with a high concentration of virus in the manufacturing pool, testing for B19 DNA by NAT in a minipool format was implemented by the manufacturer to exclude use of plasma donations with high virus titers so that the viral load in the manufacturing pool can be limited to less than 10^4 geq per mL B19 DNA.[29,32] To reduce the potential risk of transmission by other plasma-derived products, the FDA has since proposed a limit of less than 10^4 IU per mL for manufacturing pools destined for all plasma derivatives.[32-35] It is neither feasible to exclude all B19 DNA-positive plasma donations nor desirable to remove the high-titer antibody donations associated with low-level viremia.[7] Hence, highly sensitive B19 NAT assays are not suitable for this application because they hold the possibility of removing low viremic, but not infectious, plasma donations and thereby compromising B19 antibody levels in the manufacturing pool.

Some fractionators, mostly those who use source plasma, have begun to use less sensitive, or so-called high-titer, minipool NAT screening. The sensitivity of these screening tests varies with donations identified as B19-positive ranging from at least 10^5 to at least 10^7 geq per mL, but they offer a mechanism by which the viral load in manufacturing pools can be limited.[6,34-36] Some final products obtained from minipool-screened plasma have found to be devoid of B19 DNA contamination.[6]

In this study, AHF lot B was derived solely from plasma tested by a high-titer B19 NAT screening procedure and had no detectable B19 DNA. In contrast, AHF lot A was mostly derived from unscreened plasma. The transmission case might not have occurred had B19 NAT screening been performed. That is, if donations with high levels of B19 DNA had been identified, the high-titer plasma pool for the implicated lot, 10^7 geq per mL, would not have existed. A B19 transmission by a similar S/D-treated, immunoaffinity-purified, AHF product to a seronegative child with mild hemophilia A, who had not been previously infused with any blood product, has been documented.[16] As in most reported cases, however, sequencing analysis was not performed and the amount of B19 DNA infused was unknown.

Little is known regarding the correlation between a product's infectivity and its B19 DNA content. The B19 infectious dose in susceptible individuals, that is, presumably seronegative persons, would be expected to vary depending on whether the product contained anti-B19 IgG antibodies. For example, pooled plasma, S/D-treated, had levels of anti-B19 IgG[11,29] approximately 40 IU per mL in every product lot because each pool of plasma represented up to 2500 plasma donations. Only those seronegative volunteers infused with a 200-mL dose of product lots containing greater than 10^7 geq per mL B19 DNA were infected, whereas those infused with an equal volume of lots containing less than 10^4 geq per mL did not seroconvert.[29,30,32]

In a separate transmission case, a seronegative child was infected by infusing a dry heat–treated FVIII concentrate, which contained 4×10^3 geq per mL B19 DNA, over a period of 52 days.[17] The total infectious dose for this case was equivalent to 4×10^6 geq of B19 DNA from a product whose anti-B19 content, if any, was unknown. In our study, the seronegative recipient was infected by receiving a total of 2×10^4 geq of B19 DNA from a product that contained no detectable B19 IgG.

In conclusion, we have confirmed B19 transmission in a recipient of a S/D-treated high-purity AHF product derived from mostly B19 NAT unscreened plasma. The seronegative recipient became infected after receiving 2×10^4 geq (or IU) of B19 DNA present in the product.

Therefore, to safeguard the viral safety with respect to B19, minipool screening by B19 NAT should be implemented to reduce the level of potentially infectious B19 virus in the resulting products, especially those without the presence of anti-B19.

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REFERENCES

1. Young NS, Brown KE. Mechanisms of disease: parvovirus B19. N Engl J Med 2004;350:586-97.
2. Anderson MJ, Tsou C, Parker RA, et al. Detection of antibodies and antigen of human parvovirus B19 by enzyme-linked immunosorbent assay. J Clin Microbiol 1986;24:522-6.
3. Cohen BJ, Buckley MM. The prevalence of antibody to human parvovirus B19 in England and Wales. J Med Microbiol 1988;25:151-3.
4. Jordan J, Tiangco B, Kiss J, Koch W. Human parvovirus B19: prevalence of viral DNA in volunteer blood donors and clinical outcomes of transfusion recipients. Vox Sang 1998;75:97-102.
5. Aubin JT, Defer C, Vidaud M, Maniez MM, Flan B. Large-scale screening for human parvovirus B19 DNA by PCR. application to the quality control of plasma for fractionation. Vox Sang 2000;78:7-12.
6. Weimer T, Streichert S, Watson C, Gröner A. High-titer screening PCR: a successful strategy for reducing the parvovirus B19 load in plasma pools for fractionation. Transfusion 2001;41:1500-4.
7. Siegl G, Cassinotti P. Presence and significance of parvovirus B19 in blood and blood products. Biologicals 1998;26:89-94.
8. Luban NLC. Human parvoviruses: implications for transfusion medicine. Transfusion 1994;34:821-7.
9. Saldanha J, Minor P. Detection of human parvovirus B19 DNA in plasma pools and blood products derived from these pools: implications for efficiency and consistency of removal of B19 DNA during manufacture. Br J Haematol 1996;93:714-9.
10. Eis-Hübinger AM, Sasowski U, Brackmann HH. Parvovirus B19 DNA contamination in coagulation factor VIII products. Thromb Haemost 1999;81:476-7.
11. Schmidt I, Blümel J, Seitz H, Willkommen H, Löwer J. Parvovirus B19 DNA in plasma pools and plasma derivatives. Vox Sang, 2001;81:228-35.
12. Azzi A, Morfini M, Mannucci PM. The transfusion-associated transmission of parvovirus B19. Transfus Med Rev 1999;13:194-204.
13. Azzi A, Ciappi S, Zakvrzewska K, et al. Human parvovirus B19 infection in hemophiliacs first infused with two high-purity, virally attenuated factor VIII concentrates. Am J Hematol 1992;39:228-30.
14. Yee TT, Cohen BJ, Pasi KJ, Lee CA. Transmission of symptomatic parvovirus B19 infection by clotting factor concentrate. Br J Haematol 1996;93:457-9.
15. Santagostino E, Mannucci PM, Gringeri A, et al. Transmission of parvovirus B19 by coagulation factor

concentrates exposed to 100°C heat after lyophilization. Transfusion 1997;37:517-22.
16. Matsui H, Sugimoto M, Tsuji S, et al. Transient hypoplastic anemia caused by primary human parvovirus B19 infection in a previously untreated patient with hemophilia transfused with a plasma-derived, monoclonal antibody-purified factor VIII concentrate [case report]. J Pediatr Hematol Oncol 1999;21:74-6.
17. Blümel J, Schmidt I, Effenberger W, et al. Parvovirus B19 transmission by heat-treated clotting factor concentrates. Transfusion 2002;42:1473-81.
18. Schosser R, Keller-Stanislawski B, Nübling M, Löwer J. Causality assessment of suspected virus transmission by human plasma products. Transfusion 2001;41:1020-9.
19. Saldanha J, Lelie N, Yu MW, Heath A: B19 Collaborative Study Group. Establishment of the first World Health Organization International Standard for human parvovirus B19 DNA nucleic acid amplification techniques. Vox Sang 2002;82:24-31.
20. Blood safety monitoring among persons with bleeding disorders—United States, May 1998-June 2002. MMWR Morb Mortal Wkly Rep 2003;51:1152-4.
21. Shade RO, Blundell MC, Cotmore SF, Tattersall P, Astell CR. Nucleotide sequence and genome organization of human parvovirus B19 isolated from the serum of a child during aplastic crisis. J Virol 1986;58:921-36.
22. Blundell MC, Beard C, Astell CR. In vitro identification of a B19 parvovirus promoter. Virology 1987;157:534-8.
23. Bhattacharyya SP, Tan D, Guo ZP, e tal. Presence of human parvovirus B19 DNA in factor VIII concentrates: effects of viral clearance and product purification procedures. Haemophilia 2000;6:353.
24. Blümel J, Schmidt I, Willkommen H, Löwer J. Inactivation of parvovirus B19 during pasteurization of human serum albumin. Transfusion 2002;42:1011-8.
25. Yunoki M, Tsujikawa M, Urayama T, et al. Heat sensitivity of human parvovirus B19. Vox Sang 2003;84:164-9.
26. Burnouf T, Radosevich M. Nanofiltration of plasma-derived biopharmaceutical products. Haemophilia 2003;9:24-37.
27. Servant A, Laperche S, Lallemand F, et al. Genetic diversity within human erythroviruses: identification of three genotypes. J Virol 2002;76:9124-34.
28. Dorsch S, Kaufmann B, Schaible U, et al. The VP1-unique region of parvovirus B19: amino acid variability and antigenic stability. J Gen Virol 2001;82:191-9.
29. Blood Products Advisory Committee. Nucleic acid testing of blood donors for human parvovirus B19 [Internet]. Rockville (MD): U.S. Food and Drug Administration; 1999. Available from: http://www.fda.gov/ohrms/dockets/ac/99/transcript/3548t1.rtf
30. Davenport R, Geohas G, Cohen S, et al. Phase IV study of PLAS+ SD: hepatitis A (HAV) and parvovirus B19 safety results. Blood 2000;96:451a.
31. Koenigbauer U, Eastlund T, Day JW. Clinical illness due to parvovirus B19 infection after infusion of solvent/detergent-treated pooled plasma. Transfusion 2000;40:1203-6.
32. Brown KE, Young NS, Alving BM, LH.Barbosa. Parvovirus B19: implications for transfusion medicine, summary of a workshop. Transfusion 2001;41:130-5.
33. Tabor E, Yu MW, Hewlett I, Epstein JS. Summary of a workshop on the implementation of NAT to screen donors of blood and plasma for viruses. Transfusion 2000;40:1273-5.
34. Hewlett IK, Yu MW, Epstein JS. Implementation of donor screening for infectious agents transmitted by blood by nucleic acid technology. The International Forum. Vox Sang 2002;82:87-111.
35. Tabor E, Epstein JS. NAT screening of blood and plasma donations: evolution of technology and regulatory policy. Transfusion 2002;42:1230-7.
36. Aberham C, Pendl C, Gross P, Zerlauth G, Gessner M. A quantitative, internally controlled real-time PCR assay for the detection of parvovirus B19 DNA. J Virol Methods 2001;92:183-91. ◘