Reemergence of Endemic Chikungunya, Malaysia

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Chikungunya virus infection recently reemerged in Malaysia after 7 years of nondetection. Genomic sequences of recovered isolates were highly similar to those of Malaysian isolates from the 1998 outbreak. The reemergence of the infection is not part of the epidemics in other Indian Ocean countries but raises the possibility that chikungunya virus is endemic in Malaysia.

Chikungunya, a mosquitoborne disease first described in Tanzania (formerly Tanganyika) in eastern Africa in 1952, is caused by chikungunya virus (CHIKV), an alphavirus belonging to the *Togaviridae* family. The disease occurs in Africa and various parts of Asia and is endemic in several southeast Asian countries, including Thailand, Indonesia, and the Philippines. Only 1 known outbreak has occurred in Malaysia, in 1998–1999 when ≥51 persons in Port Klang were infected (*1*).

From March through April 2006, an outbreak of CHIKV infection was reported in Bagan Panchor (4°31′N, 100°37′E), an isolated coastal town 50 km west of Ipoh, the state capital of Perak, in northwest Malaysia. At least 200 villagers were infected, with no deaths reported. This was the second known outbreak in Malaysia, 7 years after the previous one. This reemergence coincided with reports of ongoing epidemics of CHIKV infection in India and almost all the island nations of the Indian Ocean, with >200,000 cases in the French island of Reunion alone since February 2005 (*2*).

Why and how the recent infection reappeared in Malaysia remains unknown. The apparent absence of CHIKV for 7 years may be due to failure to detect low-level, continued transmission in humans, particularly because the symptoms may be mistaken for dengue fever. Alternatively, this outbreak could have originated from a viremic traveler from an endemic country (such as neighboring Thailand or Indonesia), but proximity of Malaysia to the Indian Ocean raises the possibility of an extension of the epidemic, with Malaysia being the furthest point yet of the expanding epidemic frontline.

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The Study

We received serum samples from 11 patients who had symptoms typical of CHIKV infection (Table). Samples were injected into Vero and C6/36 mosquito cells. Indirect immunofluorescence assays for immunoglobulin M (IgM) and IgG were performed using the patients' sera and CHIKV-infected cells fixed onto glass slides, as previously described (*1*). A CHIKV isolate (SM287) reported previously (*3*) was used to prepare the slides as a positive control for subsequent studies. Serum samples from patients who did not have symptoms of chikungunya, including patients with dengue fever, were used as negative controls. Nucleic acid amplification was performed using RNA extracted directly from the patients' sera or from cell cultures (Table). At least 3 different primer pairs specific for envelope glycoprotein E1 (E1), glycoprotein E2 (E2), and nonstructural protein 1 (nsP1) genes of CHIKV were used (*4,5*). Confirmation of the amplified DNA fragments was done by DNA sequencing. Phylogenetic relationships were examined using the E1, E2, and nsP1 gene sequences of the isolates and all other available CHIKV sequences obtained from GenBank or the previous studies (online Appendix Table, available from www.cdc.gov/ncidod/EID/13/1/147-appT.htm). Sequences were aligned and phylogenetic trees were drawn as previously described (*6*).

CHIKV infection was confirmed in 8 of 11 patients. CHIKV sequences were amplified directly from serum samples from 5 patients in the acute phase of disease. Of these, 4 CHIKV isolates were eventually cultured. IgM and IgG were detected in serum samples from 3 other patients in the convalescent phase (data not shown). In 1 patient, CHIKV sequences were amplified from serum samples obtained as late as 9 days after onset of symptoms (data not shown). The PCR amplification method, thus, could be useful for early detection of CHIKV infection in suspected outbreak situations.

The genomic sequence of the E1, E2, and nsP1 genes in the CHIKV isolates shared high similarity (>90%) to all the known CHIKV except West African CHIKV (≈86% similarity). The sequences were only ≈70% related to o'nyong-nyong virus, the most closely related alphavirus, which is present only in certain parts of Africa. Previous phylogenetic studies showed that CHIKV strains were clustered into 3 distinct groups based on origin from West Africa, Central/East Africa, or Asia (*7–13*). Phylogenetic trees drawn using E1 (Figure), E2, and nsP1 (data not shown) gene sequences clustered the recent Malaysian isolates into a group with other known CHIKV Asian isolates. The cluster, however, was distinctly separated (100% bootstrap support) from the African isolates and all the known isolates of the ongoing CHIKV epidemics of the Indian Ocean islands (*7–9,11,13*). This makes it unlikely that the outbreak in Malaysia is part of the ongoing epidemics,

Table. Identification of virus by PCR amplification and serologic analysis*

Patient		Chikungunya						Dengue fever	
		PCR†			Serology				Serology
Age (y)	Sex	E1	E2	nsP1	IgM	IgG	Culture	PCR‡	IgM
6	M	+	+	+	–	–	+§	–	–
34	M	+	+	+	–	–	+¶	–	–
40	M	+	+	+	–	–	+#	–	–
26	F	+	+	+	–	–	+**	ND	–
62	M	+	+	+	–	–	–††	ND	–
			(day 5 after onset)						
		–	–	–	+	+	ND	ND	ND
			(day 15 after onset)						

*IgM, immunoglobulin M; IgG, immunoglobulin G; +, positive; –, negative; ND, not determined.
†PCR amplifications were performed for detection of envelope glycoprotein E1 (E1), glycoprotein E2 (E2), and nonstructural protein 1 (nsP1) genes of chikungunya virus.
‡Multiplex PCR amplifications were performed for detection of dengue virus type 1–4.
§Isolate MY/0306/BP37348.
¶Isolate MY/0306/BP37350.
#Isolate MY/0306/BP37352.
**Isolate MY/0406/BP37437.
††Isolate MY/0306/BP34198.

despite its proximity to the region and timing of the outbreak. The phylogenetic tree, on the other hand, suggests that the isolates from the current Malaysia outbreak share a common ancestral lineage to the 2 Malaysian isolates recovered in 1998 (*4*; GenBank accession nos. AF394210 and AF394211) but have a slight genetic distance from all other Asian isolates.

Conclusions

On the basis of all available sequences of isolates from the neighboring countries where CHIKV is endemic, Thailand and Indonesia, the outbreak in Malaysia likely did not originate from either of these countries, which means the outbreak could have originated from an endemic CHIKV cycle not previously identified in Malaysia. A serologic survey of human serum samples collected during 1965–1969 in west Malaysia showed neutralizing antibodies to CHIKV among adults, especially those inhabiting the rural northern and eastern states bordering Thailand (*14*). The same authors also reported in an earlier study evidence of CHIKV-neutralizing antibodies in wild monkeys, a pig, and a chicken and suggested that a CHIKV sylvatic transmission cycle involving primates and possibly nonprimates exists in Malaysia. A sylvatic transmission cycle of the virus has been described in Africa and may play a role in the episodic emergence and reemergence of CHIKV infection (*15*). Before 1998, CHIKV had not been isolated from humans or animals in Malaysia, and no clinical disease caused by CHIKV had been reported. However, in the absence of active surveillance since the 1965 study, whether the apparent absence of CHIKV over the years and between the 2 recent outbreaks in Malaysia is due to an unidentified sylvatic transmission cycle or silent transmission among humans cannot be determined. Further investigation is required to examine these possibilities. Understanding this disease in Southeast Asia is critical because CHIKV shares the same mosquito vectors as dengue virus, which is endemic to the region.

Phylogenetic analysis showed that CHIKV from the recent 2006 outbreak in Malaysia is highly similar to isolates from the 1998 outbreak. At the 3 genes examined,

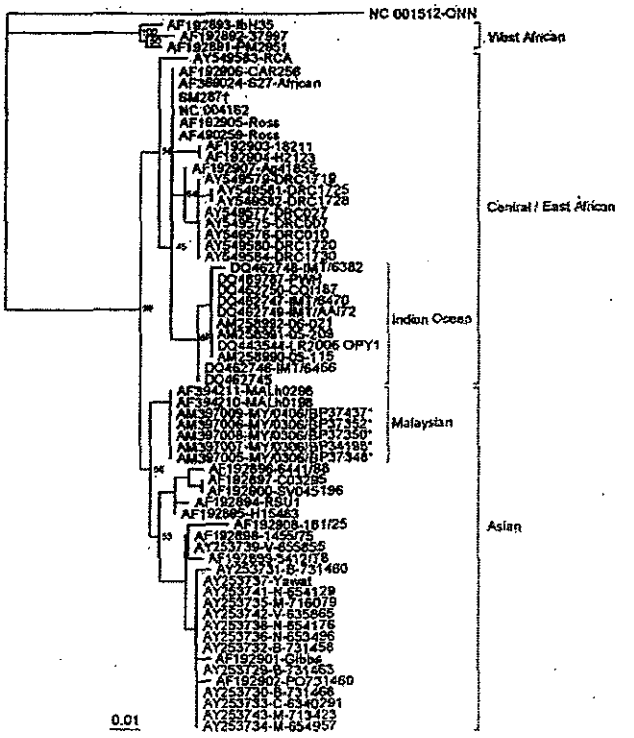

Figure. Phylogenetic relationships of chikungunya virus isolates from the 2006 Malaysia outbreak. The neighbor-joining tree was constructed using nucleic acid sequences of the envelope glycoprotein E1 gene, with o'nyong nyong virus (GenBank accession no. NC_001512) as the outgroup virus. * indicates isolates from the Malaysia 2006 outbreak; † indicates Australia SM287. Bootstrap values are shown as percentages derived from 1,000 samplings. The scale reflects the number of nucleotide substitutions per site along the branches.

the isolates differ from the ongoing Indian Ocean epidemic isolates and known isolates from Thailand and Indonesia. These findings support the possibility that the outbreak originated from an endemic infection in Malaysia.

Acknowledgments

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医薬品 研究報告 調査報告書

識別番号・報告回数			報告日	第一報入手日	新医薬品等の区分	機構処理欄
				2007. 4. 18	該当なし	

一般的名称	人赤血球濃厚液	研究報告の公表状況	Reuters AlertNet. 2007 Apr 13.	公表国	
販売名(企業名)	赤血球M・A・P「日赤」(日本赤十字社) 照射赤血球M・A・P「日赤」(日本赤十字社) 赤血球濃厚液-LR「日赤」(日本赤十字社) 照射赤血球濃厚液-LR「日赤」(日本赤十字社)			米国、ヨーロッパ	

研究報告の概要

○シャーガス病が輸血用血液を通じて米国やヨーロッパに拡大—WHO
WHOによると、感染の数十年後に死亡する可能性もある寄生虫症、シャーガス病が、不適切な血液スクリーニングが原因でラテンアメリカから米国やヨーロッパに拡大している。
WHOはバイエル社の支援を受けて、今や「地球規模の問題」となったシャーガス病根絶のための事業を拡大している。バイエル社は250万錠のNifurtimox(販売名:Lampit)を寄贈した。これは、若者の急性症例を含め今後5年間に3万人の患者を治療できる量である。
シャーガス病に感染している人は900万人にのぼると見られ、その多くはラテンアメリカの農村部の子どもである。最近では大規模な移民の影響で米国、スペインや他の欧州諸国に広がっている。シャーガス病は感染者に臓器の腫脹を引き起こし、最終的には死亡に至る病気で、正確な死亡率は不明である。大多数の感染者は、寄生虫を媒介する大型のナンキンムシに似た吸血昆虫に噛まれた後、感染していることを知らないまま数十年の潜伏期間を過ごすことになる。
「この病気はラテンアメリカの多くの人にとっては今でも脅威である。感染した供血者の適切なスクリーニングが行われていないため、血液銀行を通して脅威は他の国々に広がっている」とWHOの南北アメリカ担当者は話している。ジュネーブのWHO本部は、シャーガス病の撲滅に力を入れており、感染者数は1990年の1600～1800万人から減少してきた。
チリ、ウルグアイ、ブラジルの大部分、中米・アルゼンチン・ボリビア・パラグアイの広範囲の地域では、感染伝播は減少している。最も流行している地域は、ボリビアとアルゼンチンのチャコ地方、メキシコの一部、ペルー、コロンビアである。

報告企業の意見	今後の対応
シャーガス病が、ラテンアメリカから移住した人の供血を通して米国やヨーロッパに拡大しているとの報告である。	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。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

赤血球M・A・P「日赤」
照射赤血球M・A・P「日赤」
赤血球濃厚液-LR「日赤」
照射赤血球濃厚液-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

ıagas spread to U.S., Europe via blood banks -WHO

Apr 2007 13:47:09 GMT

urce: Reuters

NEVA, April 13 (Reuters) - Chagas, a parasitic disease which can kill victims decades after infection, has spread from Latin America to the ited States and Europe due to inadequate blood screening, the World Health Organisation said on Friday.

: United Nations agency said it was expanding its programme to eliminate Chagas, which has become a "global problem", with the help of /er HealthCare <BAYG.DE>.

/er's donation of 2.5 million tablets of Lampit, known generically as nifurtimox, will help treat an estimated 30,000 patients over the next five rs, covering new acute cases among youngsters, it said.

ıgas, which currently affects an estimated nine million people, mainly children in rural areas of Latin America, has emerged in the United tes, Spain and several other European countries after large-scale migrations, the WHO said.

exact death toll exists for the "silent killer" which causes the slow swelling of victims' internal organs, resulting in their eventual death, ording to the WHO.

st victims may not know they have contracted Chagas as the infection may remain dormant for decades after they have been bitten by a od-sucking insect similar to a large bed bug which transmits the parasite.

ıis disease still poses a threat to so many people in Latin America and now that threat has spread to other countries via blood banks lacking quate screening of infected donors," said Mirta Roses Periago, WHO director for the Americas region.

: Geneva-based WHO has been working to wipe out the disease and the number of those infected has fallen from 16-18 million people in 0.

nsmission of the disease has been interrupted in Chile, Uruguay, a large part of Brazil, as well as vast areas of Central America, Argentina, ivia and Paraguay, the WHO said.

: most endemic regions remain the Chaco regions of Bolivia and Argentina, as well as parts of Mexico, Peru and Colombia, according to ain.

REUTERS

医薬品
医薬部外品　　研究報告　調査報告書
化粧品

識別番号・報告回数			報告日	第一報入手日 2007 年 5 月 7 日	新医薬品等の区分 該当なし	厚生労働省処理欄
一般的名称	人ハプトグロビン		研究報告の公表状況	Transfusion 2007; 47(5): 883-889	公表国 アメリカ	
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研究報告の概要

＜背景＞
特に凝固因子でのパルボウイルス B19（B19）は一般的な汚染物質である。1999 年に SD 処理を施したプール血漿による B19 伝播を理由として、幾つかの分画メーカーは製造プールの B19 負荷を制限するためにミニプール NAT を開始した。本研究では、B19 NAT スクリーニングが実施された前と後で製造された市販の第Ⅷ因子（AHF）製剤中の B19 DNA 汚染の程度を確認した。
＜研究デザイン及び方法＞
1993-1998 年及び 2001-2004 年の間に製造された 6 つの AHF 製剤を代表する全部で 284 ロットを、in-house の NAT 法により B19 DNA を測定した。抗 B19 抗体（IgG）も併せて測定した。
＜結果＞
1993-1998 年に製造されたほとんどのロットから B19 DNA が検出された。陽性率は 56～100％で、製造業者により異なっていた。検出された B19 DNA の最高濃度は 10^6 IU/mL であった。検査された 40％のロットは 10^3 IU/mL であった。対照的に、2001-2004 年の間に製造された原料血漿由来の AHF 製剤の陽性率及び濃度は低かった。しかしながら、回収血漿由来の製剤では変化が見られず、これはミニプール NAT が実施されていなかったためである。中間的な精製度の AHF 製剤のみが、抗 B19 抗体陽性であった。
＜結論＞
B19 NAT をスクリーニングしていない血漿から調製した AHF の B19 DNA の陽性率及びレベルは高かったが、製造方法が異なると、製剤間でさまざまであった。血漿中の B19 NAT スクリーニングは、最終製剤中の B19 DNA レベルを下げて大半の例で検出限界以下とさせ、このことで B19 伝播のリスクを減少させた可能性がある。

使用上の注意記載状況・その他参考事項等

1. 慎重投与
(4) 溶血性・失血性貧血の患者〔ヒトパルボウイルス B19 の感染を起こす可能性を否定できない。感染した場合には、発熱と急激な貧血を伴う重篤な全身症状を起こすことがある。〕
(5) 免疫不全患者・免疫抑制状態の患者〔ヒトパルボウイルス B19 の感染を起こす可能性を否定できない。感染した場合には、持続性の貧血を起こすことがある。〕
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。
5. 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	今後の対応
原料血漿におけるミニプール NAT の実施は、最終製剤中の B19 DNA レベルを下げ、B19 伝播のリスクを減少させる可能性があるとの報告である。 本ウイルスは血漿分画製剤の製造工程での不活化・除去が困難であり、その伝播リスクを完全に否定できないため、1996 年 11 月より、使用上の注意に本ウイルスの伝播リスクについての記載を行い、注意喚起を図っている。また、原料への本ウイルス混入量低減のため、RHA(Receptor-mediated Hemagglutination)法を用いたドナースクリーニングによる高力価血漿の排除を行なっている。	本報告は本剤の安全性に影響を与えないと考えるので、特段の措置はとらない。

Parvovirus B19 DNA in Factor VIII concentrates: effects of manufacturing procedures and B19 screening by nucleic acid testing

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BACKGROUND: Parvovirus B19 (B19) is a common contaminant, especially in coagulation factors. Because of B19 transmission by pooled plasma, solvent/detergent treated in 1999, some fractionators initiated minipool nucleic acid testing (NAT) to limit the B19 load in manufacturing pools. In this study, the extent of B19 DNA contamination in commercial Factor VIII concentrates, that is, antihemophilic factor (human) (AHF), manufactured before and after B19 NAT screening was implemented, was determined.

STUDY DESIGN AND METHODS: A total of 284 lots representing six AHF products made during 1993 to 1998 and 2001 to 2004 were assayed for B19 DNA by an in-house NAT procedure. Anti-B19 immunoglobulin G (IgG) was also measured.

RESULTS: Most lots made during 1993 to 1998 had detectable B19 DNA. The prevalence ranged from 56 to 100 percent and appeared to differ between manufacturers. The highest level of B19 DNA found was 10^6 genome equivalents (geq or international units [IU]) per mL. Forty percent of the lots tested contained 10^3 geq (IU) per mL. In comparison, both prevalence and levels in source plasma–derived AHF products made in 2001 to 2004 were lower. Both, however, remained unchanged in the recovered plasma-derived product because B19 NAT screening had not been implemented. Only an intermediate-purity AHF product was positive for the presence of anti-B19 IgG.

CONCLUSION: The prevalence and levels of B19 DNA in AHF prepared from B19 NAT unscreened plasma were high but varied among products with different manufacturing procedures. B19 NAT screening of plasma effectively lowered the B19 DNA level in the final products and in the majority of cases rendered it undetectable and hence potentially reduced the risk of B19 transmission.

Parvovirus B19 (B19) is a small nonenveloped DNA virus, known to resist viral inactivation procedures commonly used in manufacturing of plasma derivatives; it is widespread among populations.[1] The prevalence of B19 viremia in blood and plasma donors has been reported to range from 0.003 to 0.6 percent, depending on the time of an epidemic or the sensitivity of nucleic acid testing (NAT) methods.[2-4] Extremely high viremic levels in plasma, for example, 10^{13} genome equivalents (geq) of B19 DNA per mL, are often found at an early phase of the infection in acutely infected but asymptomatic donors.[5] As a consequence, B19 DNA has been detected at high frequency and high levels in plasma pools and their resulting plasma derivatives, especially the coagulation products.[6-8] Reports of transmissions attributed to Factor (F)VIII concentrates

ABBREVIATIONS: AHF = antihemophilic factor (human); B19 = parvovirus B19; VI/R = viral inactivation/removal.

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The findings and conclusions in this article have not been formally disseminated by the Food and Drug Administration and should not be construed to represent any Agency determination or policy.

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(antihemophilic factor (human) [AHF]), subjected to solvent/detergent (S/D) treatment, heat treatment, or both, are numerous.[9-15]

Because of the B19 transmission in 1999 associated with pooled plasma, S/D-treated in a postmarket surveillance study that correlated product infectivity with a high concentration of virus in the manufacturing pool, plasma screening of B19 DNA by NAT in a minipool format was implemented as an in-process control test so that the viral load in the plasma pool used to manufacture the product could be limited to less than 10^4 geq per mL of B19 DNA.[16-19] The FDA has since proposed a similar limit for manufacturing pools destined for all plasma derivatives to reduce the potential risk of transmission.[19-23] Beginning in late 1999, some fractionators, mostly those who use source plasma, initiated (albeit gradually) the use of less sensitive, or so-called high-titer, minipool NAT screening to lower the viral load in manufacturing pools.[4,20-24] Some final products obtained from minipool-screened plasma have been found to be devoid of B19 DNA contamination.[4] The sensitivity of these minipool NAT tests varied but, in general, they excluded donations with B19 DNA levels of 10^6 geq per mL. In 2001, the Plasma Protein Therapeutics Association issued voluntary standards calling for manufacturers to implement 1) minipool screening of incoming plasma no later than the end of 2001 and 2) manufacturing pool testing to achieve levels of B19 DNA not to exceed 10^5 IU per mL no later than July 1, 2002.[23] Since then, all source plasma and manufacturing pools prepared from it have undergone B19 DNA testing.

The aim of this study was to evaluate the effect of B19 NAT screening of plasma on the resulting high-risk final products by comparing the prevalence and levels of B19 DNA in each of six US-licensed FVIII products made in two periods, that is, during 1993 to 1998 (before B19 NAT screening was implemented) and 2001 to 2004 (when such screening was nearly universal). Because the purification and viral inactivation/removal (VI/R) procedures used in the manufacturing of these products underwent little or no change over this entire span, the effectiveness of the B19 NAT screening could be evaluated, as could that of individual manufacturing procedures employed before any B19 screening.

MATERIALS AND METHODS

AHF samples

Six commercial AHF products represented by 136 lots made by five manufacturers during 1993 to 1998 and 148 lots made during 2001 to 2004, which were submitted by manufacturers to the FDA for lot release, were available for testing. The freeze-dried AHF products were reconstituted according to manufacturers' instructions, mostly with half of the specified volume, except that some lots made in 2004 were reconstituted with a full volume of the diluent. Unused reconstituted samples were stored at –70°C until further use.

DNA extraction and quantitation of B19 DNA by NAT

The extraction and semiquantitative NAT procedures were essentially the same as those described previously[15] except that a larger aliquot of reconstituted AHF, that is, 1.0 mL, was used for DNA extraction. For sample extraction and B19 NAT, either the WHO International Standard (NIBSC 99/800, 10^6 IU of B19 DNA/mL when reconstituted) or the CBER standard for B19 DNA (10^6 IU/mL) was used as a control.[25] Both were diluted 10^3-fold before use. Briefly, DNA from each sample or standard was extracted by use of an isolation kit and procedures (NucliSens, Organon Teknika, Durham, NC), and the DNA was recovered with 100 µL of the elution buffer. Aliquots of 25 µL of the undiluted or $10^{0.5}$-fold serially diluted DNA extracts in duplicate were used to perform nested polymerase chain reaction with primers derived from the VP1/VP2 region. Levels (in geq/mL) of B19 DNA in samples were determined by limiting dilution analysis. The sensitivity of the NAT assay for the large-volume extraction is 4 geq per mL, and the conversion ratio from geq to IU is 1:1.[15] This B19 NAT procedure detects both Genotype 1 and Genotype 2 of B19 but not the Genotype 3 variant (see Discussion).

Detection of anti-B19

Anti-B19 immunoglobulin G (IgG) was detected by use of a B19 IgG enzyme immunoassay kit (Biotrin International Ltd, Dublin, Ireland) according to the manufacturer's instructions except that a large sample aliquot, that is, 100 µL, was used for testing each reconstituted AHF. Most of the AHF samples tested were B19 DNA–positive.

Statistical analysis

The chi-square test was used to compare the prevalence between products. In addition, for comparing viral levels expressed as the log geometrical mean ± standard error of log geometrical mean (SEM), statistical analysis was performed by use of the unpaired t test. Results having p values of less than 0.05 were considered significant.

RESULTS

Prevalence and levels of B19 DNA in AHF lots manufactured during 1993 to 1998: effects of manufacturing procedures

Most products were made mainly from source plasma, but Product C was made from recovered plasma. The various purification and VI/R procedures used in the manufactur-