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HENDRA VIRUS, HUMAN, EQUINE - AUSTRALIA (03): (QUEENSLAND)
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Anxious watch over vet staff in virus outbreak

The owner of a Brisbane veterinary clinic is anxiously waiting to see if more of his staff have contracted the potentially fatal Hendra virus. A nurse and a veterinarian at the Redlands Veterinary Clinic were diagnosed with the virus after treating several infected horses. Owner Dr David Lovell said if no more staff were diagnosed this weekend [19-20 Jul 2008], the worst of the crisis should be over. "If we get through this weekend I get the feeling we will be on the road to recovery," Lovell said. "The anticipated maximum incubation period is 14 days and certainly by Tuesday [22 Jul 2008] there would be absolutely no chance of there being a human or horse being exposed or infected because everything would have been shut down and secured for that time."

Lovell said staff had visited the nurse and veterinarian Ben Cunneen in the Princess Alexandra Hospital. "They are no way near being cured but it just means they are not deteriorating and that has to be some cause for optimism. But this is not detracting one bit from the seriousness of the condition."

The veterinarian of 38 years has closed his horse practice during the crisis as 8 other staff who worked closely with affected horses are monitored to see if they are incubating the bug. One of the horses was put down, another died and a 3rd is recovering. Lovell said those horses showed signs of neurological damage such as a staggered gait and falling over.

Cunneen and the nurse suffered flu-like symptoms from the virus, which claimed the life of trainer Vic Rail and 14 horses during the last outbreak in 1994. Brisbane Southside Population Health Unit medical officer Dr Brad McCall said the affected pair would have acquired the infection through close contact with the horses in the late stage of illness or at autopsy. There had been no evidence of person to person transmission of the virus and no risk to the wider community.

Queensland Health continues to monitor 7 people in Proserpine, north Queensland, who have undergone blood tests following a 2nd outbreak of the virus. A virus-affected horse died late last week at a Cannonvale property.

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[The 1st human case of Hendra virus infection in the outbreak affecting horses at the Redlands Veterinary Clinic in Brisbane was reported on 15 Jul 2008. Now a 2nd person working at the Redlands Veterinary Clinic has been

hospitalised with Hendra virus infection. The condition of these 2 patients appears to be serious but not life-threatening.

The interactive HealthMap/ProMED-mail interactive map of Australia can be accessed at <http://healthmap.org/promed?v=-25.7,134.5,4> to find the location of the city of Brisbane in the state of Queensland. - Mod.CP]

[see also:
Hendra virus, human, equine - Australia (02): (QLD,NSW) 20080717.2168
Hendra virus, human, equine - Australia: (QLD) 20080715.2146
Hendra virus, equine - Australia: (Brisbane) 20080708.2076
2007

Hendra virus, human, equine - Australia (QLD) (04): 2nd corr. 20070903.2902
Hendra virus, human, equine - Australia (QLD) (03): corr. 20070903.2896
Hendra virus, human, equine - Australia (QLD) (02): not 20070831.2871
Hendra virus, human, equine - Australia (QLD): RFI 20070830.2851
2006

Hendra virus, equine - Australia (NSW): susp. 20061109.3222
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1999

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Hendra virus, horse - Australia (Queensland) 19990219.0218]

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研究報告の概要

＜研究デザイン及び方法＞
ヒトの8つのヘルペスウイルスの同定と定量のために新たに開発された一連のRT-PCRを利用して、テキサス南東部から無作為に抽出した100名の血液ドナーの白血球を豊富に含む血液の陽性率とウイルスDNA量を測定し報告する。
＜結果＞
単純ヘルペスウイルス1及び2型（HSV-1及びHSV-2）、水痘帯状疱疹ウイルス（VZV）、及びHHV-8 DNAは、いずれのドナーにも検出されなかった。対照的に、エプスタインバーウイルス（EBV）（72%）およびHHV-7（65%）は検出頻度が高く、HHV-6（30%）は頻繁に検出され（B型のみ）、サイトメガロウイルス（1%）はめったに検出されなかった。陽性サンプル中のウイルス量の中央値は、血液1mLあたりHHV-6の4237からEBVの46未満の範囲におよんでいた。
＜結論＞
これらの結果から、健康な成人ドナーからの輸血によるヘルペスウイルス感染の可能性は、EBV及びHHV-7で高く、HHV-6で中程度に高く、CMVでは低く、HSV-1、HSV-2、VZV及びHHV-8ではめったにないことが示唆される。本研究で最も注目に値するのは、1人のドナーの血液から6.1×10^7genome equivalent/mLを超えるHHV-6 Type Bが検出されたことである。異常に高いHHV-6 DNAのレベルが健康な成人血液ドナーから検出されたことから、この現象は活動性感染または免疫不全と関係がないようである。

使用上の注意記載状況・その他参考事項等

2. 重要な基本的注意
(1) 本剤の原材料となる献血者の血液については、HBs抗原、抗HCV抗体、抗HIV-1抗体、抗HIV-2抗体、抗HTLV-I抗体陰性で、かつALT(GPT)値でスクリーニングを実施している。更に、プールした試験血漿については、HIV-1、HBV及びHCVについて核酸増幅検査（NAT）を実施し、適合した血漿を本剤の製造に使用しているが、当該NATの検出限界以下のウイルスが混入している可能性が常に存在する。本剤は、以上の検査に適合した血漿を原料として、人血液凝固第Ⅷ因子-vWF複合体を濃縮・精製した製剤であり、ウイルス不活化を目的として、製造工程においてリン酸トリ-n-ブチル（TNBP）/ポリソルベート80処理、凍結乾燥の後、60℃、72時間の加熱処理を施しているが、投与に際しては、次の点に十分注意すること。

報告企業の意見	今後の対応
健常人血液ドナーからEBV、HHV-7、HHV-6が高頻度に検出され、またHHV-6については異常に高いレベルのウイルスが検出されたとの報告である。 万一、原料血漿にヘルペスウイルスが混入したとしても、BHV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。	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。

BENE2008-012

Herpesvirus prevalence and viral load in healthy blood donors by quantitative real-time polymerase chain reaction

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BACKGROUND: After primary infection, human herpesviruses (HHVs) maintain long-term latent persistence, often punctuated years later by sporadic episodes of symptomatic lytic activation. Also, blood-borne herpesvirus from healthy persistently infected blood donors can lead to active primary infection of immunocompromised transfusion recipients.

STUDY DESIGN AND METHODS: Utilizing a set of newly developed real-time polymerase chain reaction assays for detection and quantification of all eight human herpesviruses, the prevalence and viral DNA load of white cell–enriched blood from 100 randomly selected blood donors from the southeast Texas region are reported.

RESULTS: Herpes simplex viruses 1 and 2 (HSV-1 and HSV-2), varicella-zoster virus (VZV), and HHV-8 DNA were not detected in any donor sample. In contrast, Epstein-Barr virus (EBV) (72%) and HHV-7 (65%) were commonly detected, HHV-6 (30%) was often detected (Type B only), and cytomegalovirus (CMV; 1%) was rarely detected. Median viral loads of positive samples (per milliliter of blood) ranged from 4278 for HHV-6 to less than 46 for EBV.

CONCLUSIONS: These results suggest that the potential for transfusion-mediated transmission of herpesviruses from healthy adult blood donors is high for EBV and HHV-7; moderately high for HHV-6; uncommon for CMV; and rare for HSV-1, HSV-2, VZV, and HHV-8. Perhaps the most remarkable finding in this study was the detection of a single donor sample with greater than 6.1×10^7 HHV-6 Type B genome equivalents per mL blood. Given that this extraordinarily high level of HHV-6 DNA was obtained from a healthy adult blood donor, this phenomenon is likely unrelated to active infection or immunodeficiency.

The eight human herpesviruses (herpes simplex virus 1 and 2 [HSV-1, HSV-2], varicella-zoster virus [VZV], Epstein-Barr virus [EBV], cytomegalovirus [CMV], human herpesvirus 6 [HHV-6], human herpesvirus 7 [HHV-7], and human herpesvirus 8 [HHV-8, KSHV]) are large enveloped double-stranded DNA viruses that establish asymptomatic life-long latent persistence in host cells after primary infection.[1] Given the moderate to high seroprevalence rates for all but HHV-8, and the fact that most of the herpesviruses (EBV, CMV, HHV-6, HHV-7, HHV-8) maintain latency in white cells (WBCs), it is likely that a large number of adult blood donors carry herpesvirus DNA in whole blood.

There have been a number of excellent published studies regarding herpesvirus DNA prevalence and virus load in adult donor blood. Many of these studies, however, were performed with relatively few specimens (≤20), many did not determine viral load, and only one previous study[2] of 20 donors assayed for all eight herpesviruses.

A novel nested polymerase chain reaction (PCR) assay with a complex mixture of degenerate and deoxyinosine-substituted primers to the highly conserved herpesvirus DNA polymerase gene was previously developed for the purpose of discovery of novel herpesviruses in animals.[3] Our group adapted this general method for the detection

ABBREVIATIONS: HHV = human herpesvirus; HSV = herpes simplex virus; VZV = varicella-zoster virus.

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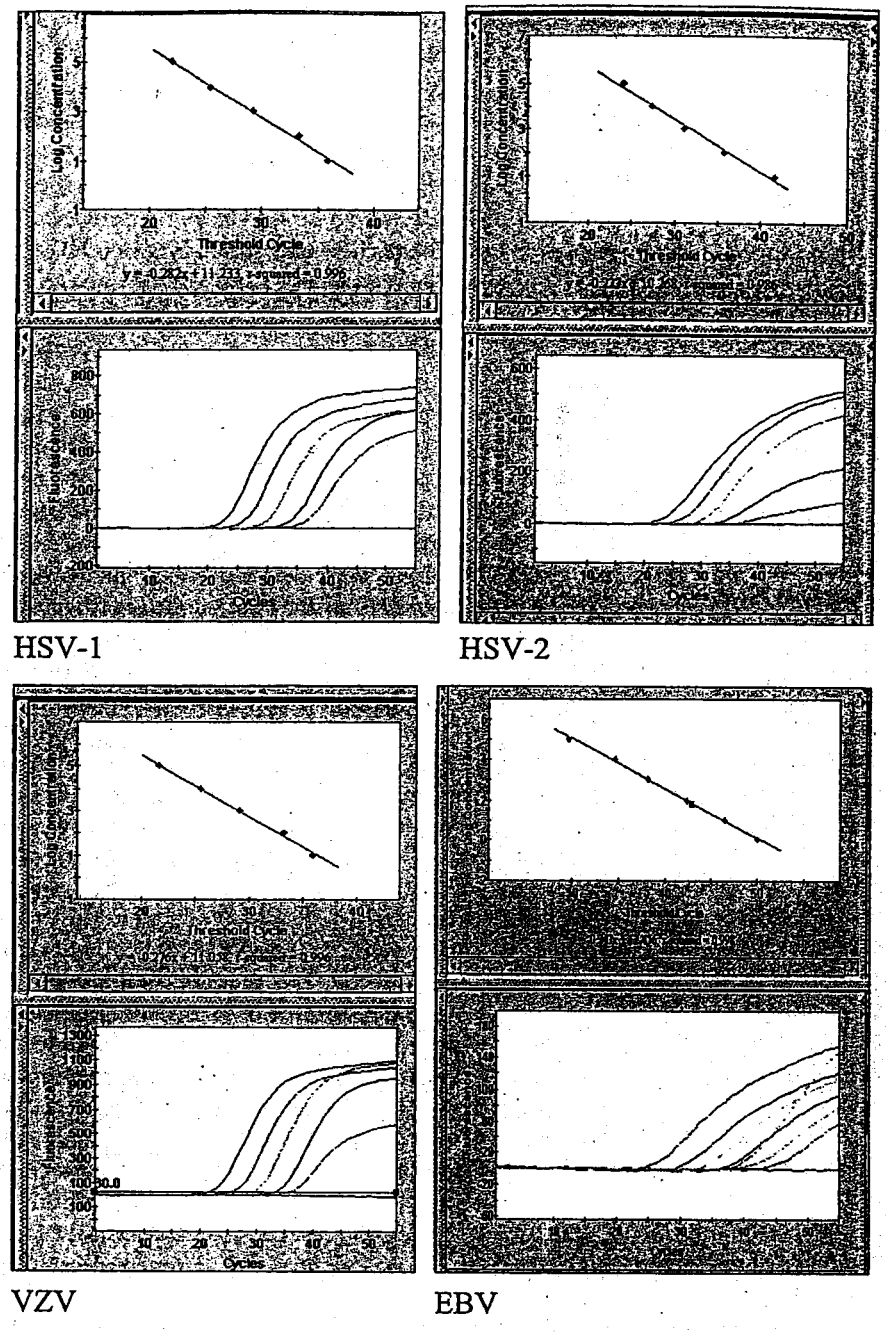

Fig. 1. Real-time PCR standard curves. The top panel displays the linear relationship between log concentration of viral DNA and PCR cycle. The bottom panel demonstrates the relationship between fluorescence signal intensity and PCR cycle. The curves from left to right in the lower panel represent serial dilutions of viral DNA—10^5, 10^4, 10^3, 10^2, and 10^1 viral geq per PCR procedure (per µg). Results for 10^6 geq are shown only for EBV, CMV, and HHV-8.

and differentiation of all eight human herpesviruses by chemiluminescent dot blot nucleic acid hybridization and heteroduplex mobility gel shift assay.[4] While these assays have proven to be excellent tools for herpesvirus detection and differentiation, they do not allow for viral load determination. To address this limitation, we have developed a set of eight real-time PCR assays with TaqMan probes for detection and quantification of the human herpesviruses and have applied these assays to determine the prevalence and viral load of herpesvirus DNA from 100 randomly selected donor blood samples.

MATERIALS AND METHODS

Real-time PCR

Herpesvirus DNA was obtained from the following sources: HSV-1 (ATCC, Rockville, MD), HSV-2 (ATCC), VZV (Ellen strain, ATCC), EBV (B95-8, ATCC), CMV (AD169 strain, ATCC), HHV-6 (U1102 Type A strain and Z29 Type B strain, Advanced Biotechnologies, Columbia, MD), HHV-7 (H7-4 strain, Advanced Biotechnologies), and HHV-8 (BCBL-1, NIH AIDS Reagent Program, Rockville MD). PCR products of each herpesvirus obtained by regular PCR (*Taq* polymerase, Sigma, St Louis, MO) were agarose gel–purified, cloned into the TOPO TA cloning vector (Invitrogen, Carlsbad, CA), and confirmed by DNA sequencing. Herpesvirus plasmid DNA was quantified by ultraviolet (UV) spectrophotometry (DU 640, Beckman, Fullerton, CA) and stored frozen at –20°C until use.

Assay specificity was determined by simultaneously performing two PCR procedures for each set of primers. One reaction was performed with a control sample containing DNA of all eight herpesviruses as template (positive control), and the other reaction was performed with a control sample containing DNA of all but the primer-specific virus (negative control). In each case (data not shown), all primer sets yielded a positive product with the positive control and no product with the negative control. Assay sensitivity was determined with six serial 10-fold dilutions (10^5-10^0 virus genome equivalents [geq]) of each herpesvirus plasmid DNA prepared in TE buffer (10 mmol/L Tris-HCl, 1 mmol/L ethylenediaminetetraacetate, pH 8.0). The standard curves for each virus are displayed in Fig. 1. Linearity of all log standard curves was excellent, with $r^2 > 0.98$ for all eight assays. The limits of detection (sensitivity) of each assay are as follows: HSV-1, 10 geq per µg DNA; HSV-2, 10 geq;

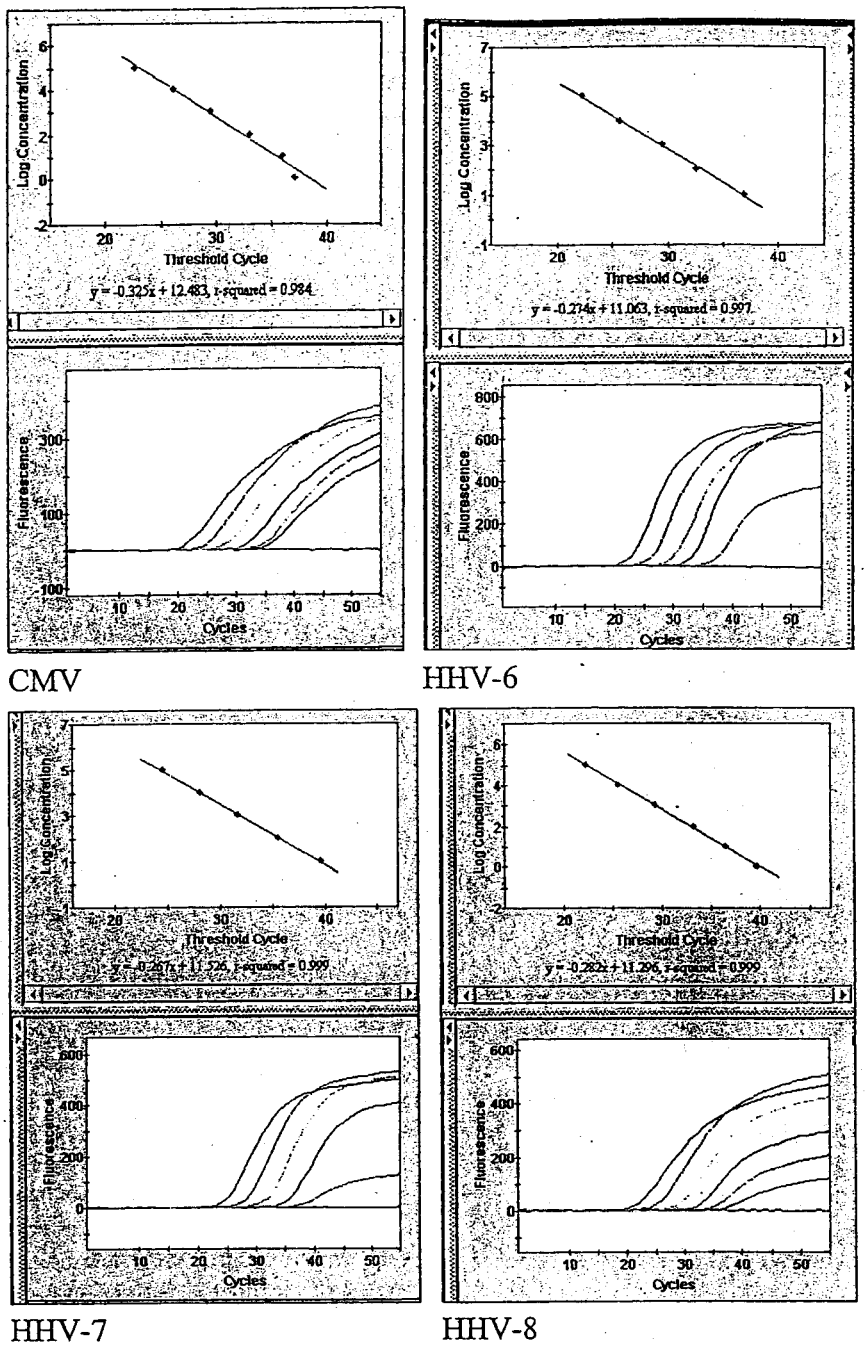

Fig. 1. *Continued*

VZV, 10 geq; EBV, 1 geq; CMV, 1 geq; HHV-6, 5 geq; HHV-7, 10 geq; and HHV-8, 1 geq.

DNA was extracted from 100 samples of WBC-rich whole blood obtained from the Gulf Coast Regional Blood Center (Houston, TX) with a DNA mini kit (QIAamp, Qiagen, Valencia, CA), quantified by UV spectrophotometry (DU 640, Beckman), and stored frozen in TE buffer at –20°C until use.

One-step real-time PCR assays for all eight herpesviruses were first developed. These single-step assays proved to be sufficiently sensitive for detection of all herpesviruses except for EBV and HHV-6. Because single-step assays for EBV and HHV-6 proved to be less sensitive in detection of low viral copy number, we developed nested PCR assays for detection of small quantities (<1000 geq/μg) of EBV and HHV-6 (Fig. 1).

To ensure that the nested PCR procedures were quantitative, standard curves for both stages of amplification with high viral load standards were constructed. We were careful to limit the first amplification step (with external primers) to 20 cycles, a cycle number empirically chosen based on results of single-step real-time PCR in which samples with viral loads as high as 2×10^6 copies per mL reverted to positive only after more than 20 cycles of amplification (as shown in Figs. 1 and 2). In addition, standard curves for the nested PCR clearly indicated that the assay was log-linear and quantitative for high viral load samples (Fig. 2).

One microgram of sample DNA (or 2 μL of external EBV and HHV-6 PCR products) was added to a real-time PCR tube containing 12.5 μL of 2× ready mix (JumpStart *Taq*, Sigma), 0.3 μmol per L primers, 0.2 μmol per L dual-labeled probes, 5 mmol per L $MgCl_2$, and ultrapure water up to 25 μL final volume. Real-time PCR was performed in a rapid thermal cycler (Smart Cycler, Cepheid, Sunnyvale, CA) machine under the following conditions: 95°C for 2 minutes, followed by 45 to 55 amplification cycles of 95°C for 15 seconds, 60°C (50°C for HHV-6) for 30 seconds, and 72°C for 30 seconds. All TaqMan primers and probes (see Appendix S1, available online at http://www.blackwell-synergy.com/doi/abs/10.1111/j.0041-1132.2008.01685.x) were produced by Sigma Genosys (The Woodlands, TX) and tested for sensitivity and specificity.

For external EBV PCR, a 1-μg sample of DNA was added to a PCR tube containing 3 μL of 10× reaction buffer (200 mmol/L Tris-HCl, pH 8.8, 100 mmol/L KCl, 100 mmol/L $(NH_4)_2SO_4$, 20 mmol/L MgCl2, 1% Triton X-100, 1 mg/mL bovine serum albumin), 1.2 μL of 25 mmol per L $MgCl_2$, 0.6 μL of 10 mmol per L dNTP mix, 1.5 units of *Taq* polymerase (Orbigen, San Diego, CA), 6 μL of 5× CES (2.7 mol/L betaine, 6.7 mmol/L dithiothreitol,

6.7% dimethyl sulfoxide, and 55 µg per mL bovine serum albumin), 0.3 µmol per L external primers (Set 1), and ultrapure water up to 30 µL final volume. External EBV PCR was performed in a conventional thermal cycler (Peltier, PTC-200, MJ Research, South San Francisco, CA) under the following conditions: 95°C for 2 minutes and 20 amplification cycles of 95°C for 30 seconds, 56°C for 40 seconds, and 72°C for 1 minute, followed by a final 6 minutes' extension at 72°C. EBV internal nested PCR was performed with internal primers (Set 2) and 0.2 µmol per L dual-labeled probe.

For external HHV-6 PCR, 1 µg of sample DNA was added to a preloaded PCR tube (EasyStart micro50, Molecular BioProducts, San Diego, CA) to which was added 5 µL of 1 percent Triton X-100, 2.5 units of *Taq* polymerase (Orbigen), 3 µL of 25 mmol per L $MgCl_2$, 0.32 µmol per L of external primers (Set 1), and ultrapure water up to 50 µL final volume. External HHV-6 PCR was performed in a conventional Peltier thermal cycler (PTC-200, MJ Research) under the following conditions: 94°C for 2 minutes and 20 amplification cycles of 94°C for 30 seconds, 60°C for 30 seconds, and 72°C for 30 seconds, followed by a final 5 minutes' extension at 72°C. HHV-6 internal nested PCR was performed with internal primers (Set 2) and both HHV-6A and HHV-6B type–specific probes (0.2 µmol/L each). For extremely high viral loads (as seen with Case 46), our experience indicates that the use of two PCR procedures, each with a single HHV-6 type–specific probe, is preferable.

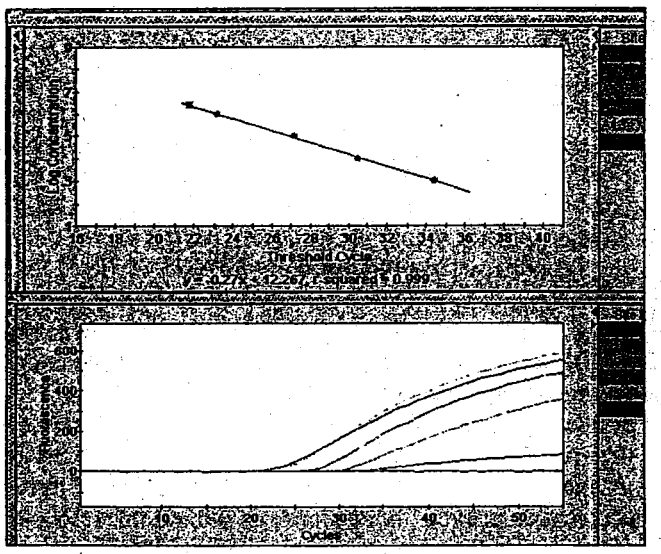

Fig. 2. Real-time PCR standard curve for HHV-6 high-viral-load samples. PCR positivity of extremely high HHV-6 viral loads (>10^6 copies/reaction) was seen only after more than 20 cycles of single-step PCR and yields a highly linear log standard curve with a range of 10^5 to 2.45×10^6 virus copies per reaction. The linearity of the assays allows for viral load quantification of samples with high viral load by one-step PCR.

Virus load calculation

Because each human diploid cell contains approximately 6.6 pg DNA, 1 µg of human genomic DNA from blood was derived from approximately 1.5×10^5 WBCs. One milliliter of whole human blood contains approximately 7×10^6 nucleated cells (WBCs). Thus, the virus copy number (geq) per milliliter of blood is equal to virus copy number per µg of DNA (as determined by the real-time PCR assay) multiplied by 47 µg of DNA per mL blood.

RESULTS

Herpesvirus DNA was commonly detected, with 94 of 100 donor blood samples positive for the presence of at least one herpesvirus (results summarized in Table 1). No herpesvirus DNA was detected in 6 cases. Four herpesviruses (HSV-1, HSV-2, VZV, HHV-8) were undetected in any sample, and CMV was detected in only a single case. In contrast, EBV (72%), HHV-7 (65%), and HHV-6 (30%) were commonly detected. All 30 cases of HHV-6 were Type B; that is, no HHV-6 Type A was identified. Median viral loads of positive samples (virus geq/mL blood) were 4,371 for HHV-6 (range 188-61,610,713), 3,196 for CMV (1 case only), 1,763 for HHV-7 (range 282-27,401), and less than 47 for EBV (range, <47-550,370). A single donor sample containing more than 80×10^6 geq of HHV-6B DNA per mL was identified. Because 1 mL of normal adult blood contains approximately 7×10^6 WBCs, this extremely high viral load translates to approximately 11 virus copies per WBC. Seventeen donor blood samples were positive for the presence of three herpesviruses (16 with EBV, HHV-6, and HHV-7; 1 with EBV, CMV, and HHV-7).

TABLE 1. Prevalence and virus load of herpesviruses in blood donors

	HSV-1	HSV-2	VZV	EBV	CMV	HHV-6	HHV-7	HHV-8
Total samples	100	100	100	100	100	100	100	100
Positive samples	0	0	0	72	1	30	65	0
Median viral load*				<47	3196	4371	1763	
Viral load range				<47-5.5×10^5		188-6.2×10^7	282-2.7×10^4	

* Expressed as virus copy number per mL of whole blood. Each PCR procedure was performed on 1 µg of whole-blood DNA, representing approximately 1.5×10^5 WBCs.

DISCUSSION

Given that acute infection with human herpesviruses may sometimes lead to serious disease, issues regarding the frequency and clinical significance of blood transfusion–mediated transmission of herpesviruses from chronically infected donors to previously uninfected or immunocompromised recipients have been raised. Although these issues have been addressed in the case of CMV, the frequency and significance of infection with the other herpesviruses have not been as thoroughly detailed.

Little information regarding the frequency and virus load of HSV-1–positive blood donors is available. HSV-1 PCR positivity was not detected in healthy adult blood donors from three independent studies.[2,5,6] With a highly sensitive real-time PCR assay, we detected no HSV-1–positive samples from a cohort of 100 adult blood donors. Our results corroborate the earlier negative reports and suggest that HSV-1 transmission by blood transfusion is likely to be a highly unusual event.

Information regarding detection of HSV-2 in healthy adult blood donors is extremely limited. In one small study,[2] HSV-2 PCR positivity was not detected in 20 adult blood donors. In the current study, we detected no HSV-2–positive samples from 100 adult blood donors. Our results corroborate the earlier negative findings and indicate that HSV-2 transmission by blood transfusion is likely to be a highly unusual event.

Relatively little information regarding the incidence of VZV DNA positivity in donor blood is available. Hoang and coworkers[2] detected only 1 VZV-positive sample (virus load 39,029 geq/mL) from a total of 20 samples, whereas de Jong and coworkers[7] detected no positive samples from a total of 20. In our study of 100 donor samples, no positive samples were identified. Thus, these data suggest that VZV transmission by donor blood is likely to be an infrequent event.

Given the very real clinical concerns with transfusion-mediated CMV transmission in immune-compromised recipients, several studies have addressed the issue of CMV positivity in donor blood. Whereas a relatively high frequency of CMV DNA positivity (19%-33%) has been described by some investigators,[5,6,8] other investigators have reported much lower rates of CMV positivity, ranging from 0 to 2.8 percent.[2,9-12] Roback and colleagues[9] identified only 2 positive samples of 1000 samples from the United States whereas Nishiwaki and coworkers[10] identified 27 positive samples of 953 samples from Japan. In the current study, we identified only 1 CMV-positive donor sample of 100 samples from the United States, a result that is consistent with the low prevalence previously reported in US blood donors.[9] In this previous report,[9] the 2 positive samples yielded an estimated 10 to 99 CMV geq per 2.5×10^5 WBCs. In an earlier article,[13] this same group reported donor blood CMV viral loads ranging from 8 to 1560 geq per 2.5×10^5 blood WBCs. Our positive sample contained 3196 CMV geq per mL of blood. Given that 1 mL of blood contains approximately 7×10^6 WBCs, our single positive case contains approximately 114 CMV genomes per 250,000 WBCs, a result remarkably similar to that previously reported.[9]

Give the role of EBV infection in the pathogenesis of posttransplant lymphoproliferative disorders, there has been a great deal of interest in determination of EBV viral load in donor blood. Although EBV infection is very common with greater than 96 percent seroprevalence in adults worldwide,[14] there is a wide range of reported rates for EBV DNA positivity of donor blood, ranging from 5 to 88 percent.[2,5,6,10,15-20] In this study, with a real-time nested PCR method, 72 percent of the donor blood samples contained EBV DNA. The sensitivity of our assay is 1 geq per μg of DNA. We suspect that the lower rates for EBV positivity reported by some investigators were obtained with less sensitive assays. Regarding EBV DNA load in blood, Hoang and colleagues[2] reported 845 geq per mL, Kimura and colleagues[15] reported 585 geq per mL (15.8 geq/μg), and Maurmann and colleagues[19] obtained a range of 3055 to 851,170 geq per mL. The current results indicate that EBV load varies over a wide range, with some donor blood samples containing more than 500,000 geq per mL, a result consistent with those previously reported by Maurmann and colleagues.[19] Qu and coworkers[20] reported the interesting observation that removal of WBCs from 14 EBV DNA–positive whole-blood units rendered all but 1 unit EBV DNA–negative. Thus, although EBV DNA positivity of whole donor blood appears to be quite common, the risk of EBV transmission from red blood cell transfusion is significantly reduced by leukoreduction.

In the current study, HHV-6 DNA was detected in 30 percent of the blood donor samples. At least six previous studies have reported rates of HHV-6 DNA positivity and virus load from adult donor blood samples. In one early study, Wilborn and colleagues[21] reported HHV-6 positivity in only 5.4 percent of donor blood (buffy coat) samples. In four later studies, HHV-6 DNA positivity was detected in 25 to 36 percent of donor blood samples.[2,22-24] Cuende and colleagues[25] made the interesting observation that using 1 μg of DNA, 40 percent of the samples were positive, a rate similar to that reported in the four previously mentioned studies, whereas using 5 μg DNA, 90 percent of the same samples were positive. Assuming that these results are not due to contamination, nonspecificity, or technical error, this finding suggests that detection of extremely low levels of virus may in some cases require amplification of larger amounts of sample DNA. It should be noted, however, that the 30 percent HHV-6 positivity rate obtained in the current study was obtained with an assay with a high sensitivity (5 geq/μg DNA).

Clearly the most surprising finding from the current study was the identification of a single blood donor

sample that contained more than 6.1×10^7 geq of HHV-6 per mL of blood. To ensure the validity of this result the assay was performed four times, with the same result obtained each time. Unusually high levels of HHV-6 DNA were first reported by Luppi and coworkers[26] in peripheral blood mononuclear cells from three patients, two with lymphoproliferative disorders and one with multiple sclerosis. The fact that two of the three patients were HHV-6–seronegative suggested that the virus infection was latent. Luppi and coworkers[26] further demonstrated that the viral genome was integrated into WBC DNA. Clark and colleagues[22] described a single healthy adult with 1.2×10^6 HHV-6 geq per μg DNA (56.4×10^6 geq/mL) in blood that persisted for at least 10 months with no evidence of active disease. These findings have been confirmed and extended by others.[27-31] Tanaka-Taya and coworkers[29] concluded that these levels of viremia translate to more than 1 virus copy per blood WBC. Ward and colleagues[31] identified six patients with a mean of 10^7 geq of HHV-6 per mL of whole blood. These six individuals, ranging in age from newborn to 58 years, presented with a variety of symptoms including neonatal convulsions, EBV-associated encephalitis, and meningitis, while one individual was a healthy adult stem cell donor. Based on demonstration of HHV-6 integration in hair follicle cells and previous reports of vertical transmission of integrated HHV-6,[28,29] Ward and colleagues[31] concluded that the virus was carried by all cells and inherited through the germline.

The current case represents to our knowledge the first report of this unusual phenomenon in a healthy adult blood donor. Because the virus appears latent and unable to provoke a humoral immune response, we believe that this phenomenon likely poses no serious risk to an immunocompetent recipient. It is most likely that in a fully immunocompetent recipient, transfused WBCs carrying latent integrated HHV-6 will be normally cleared from the recipient with no residual infected donor cells. On the other hand, the outcome in immunocompromised recipients or in those who receive stem cell transplants is less certain. In an immunodeficient patient the possibility of viral activation of latent integrated virus leading to acute virus infection cannot be absolutely excluded. Assuming that integrated virus is present in hematopoietic stem cells, it seems likely that recipients of stem cell transplants from donors that carry integrated HHV-6 will permanently carry integrated virus in their hematopoietic cells. The clinical implications of this phenomenon are not known.

HHV-7 infection, like EBV infection, is very common, with a reported seroprevalence of 96 percent.[32] In an early study, no HHV-7 DNA positivity was detected in 20 donor blood samples.[2] In a more recent study,[33] HHV-7 DNA was detected in peripheral blood mononuclear cells from 87 percent of blood donors. In the present study, HHV-7 DNA was detected in 65 percent of donor blood samples, a result similar to the previous study.[33] The earlier negative results[2] were obtained with a nonnested PCR assay coupled with gel detection of product, whereas the current results were obtained with a real time PCR assay. Because the limits of detection of the assays utilized by Hoang and colleagues[2] ranged from 222 (VZV) to 1738 (HSV-2), it is likely that the marked difference in HHV-7 DNA prevalence obtained by these studies is due to the relative insensitivity of the earlier assays.

HHV-8, the most recently discovered human herpesvirus, is also the least commonly encountered in the United States in terms of seroprevalence, with a range of less than 1 to 24 percent depending on geographic region and serologic technique.[1] In terms of HHV-8 DNA positivity of healthy adult blood donors, there is relatively little information. In two independent studies, Hudnall and colleagues[34] and Hoang and colleagues[2] identified no HHV-8 DNA positivity from an aggregate total of 40 donor whole-blood samples, and Broccolo and coworkers[35] identified no HHV-8 DNA positivity from 36 donor plasma samples. The current study extends and corroborates these negative findings by identifying no HHV-8 DNA positivity from 100 donor blood samples with a highly sensitive assay capable of detecting a single virus copy. These results indicate that HHV-8 DNA positivity of adult donor blood in the United States is likely to be a rare phenomenon.

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SUPPLEMENTARY MATERIAL

The following supplementary material is available for this article:

Appendix S1. Real-time PCR reagents (Word document).

This material is available as part of the online article from: http://www.blackwell-synergy.com/doi/abs/10.1111/j.0041-1132.2008.01685.x
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