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HENDRA VIRUS, HUMAN, EQUINE - AUSTRALIA (03): (QUEENSLAND)
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A ProMED-mail post
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Date: Sun 20 Jul 2008
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<http://www.nzherald.co.nz/section/4/story.cfm?c_id=4&objectid=10522474>

Anxious watch over vet staff in virus outbreak
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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
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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
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Hendra virus, equine - Australia (NSW): susp. 20061109.3222
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Hendra virus - Australia (QLD) 20041214.3307
1999
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Hendra virus - Malaysia, Singapore: Fact sheet 19990319.0434
Hendra virus, horse - Australia (Queensland) 19990219.0218]

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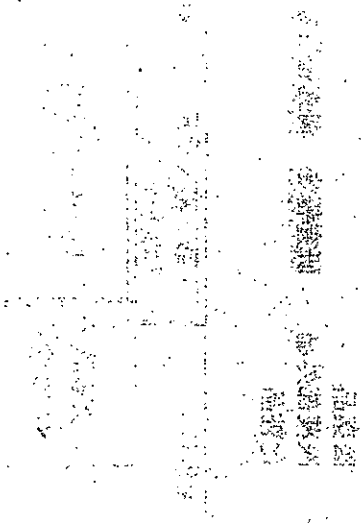
**研究報告の概要**

＜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。

| 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。<br>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。 |

**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。



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 $6.1 \times 10^7$ 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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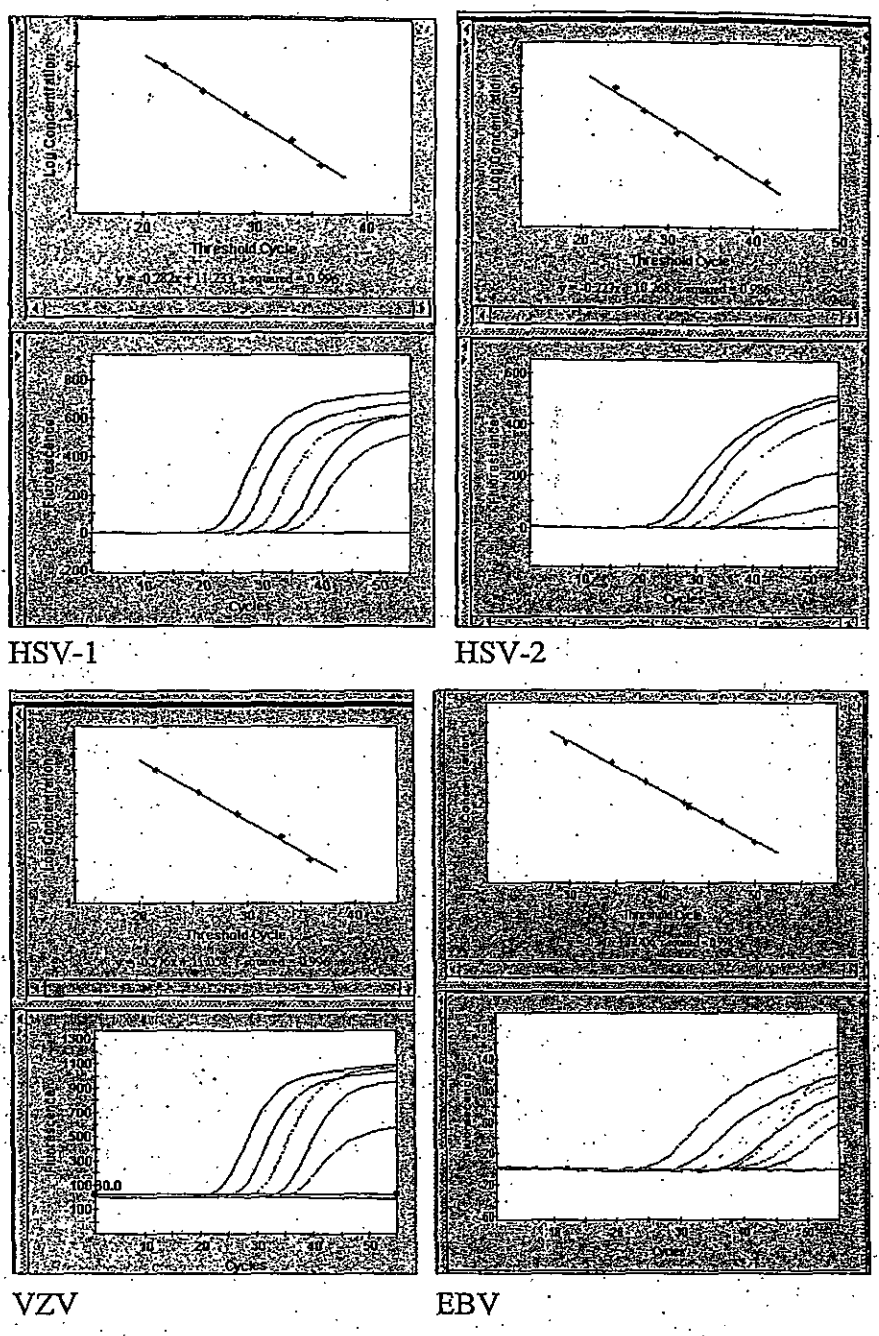


**Fig. 1. Real-time PCR standard curves. The top panel displays the linear relationship between log concentration of viral DNA and PCR cycle. The bottom panel demonstrates the relationship between fluorescence signal intensity and PCR cycle. The curves from left to right in the lower panel represent 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 $10^0$ 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;

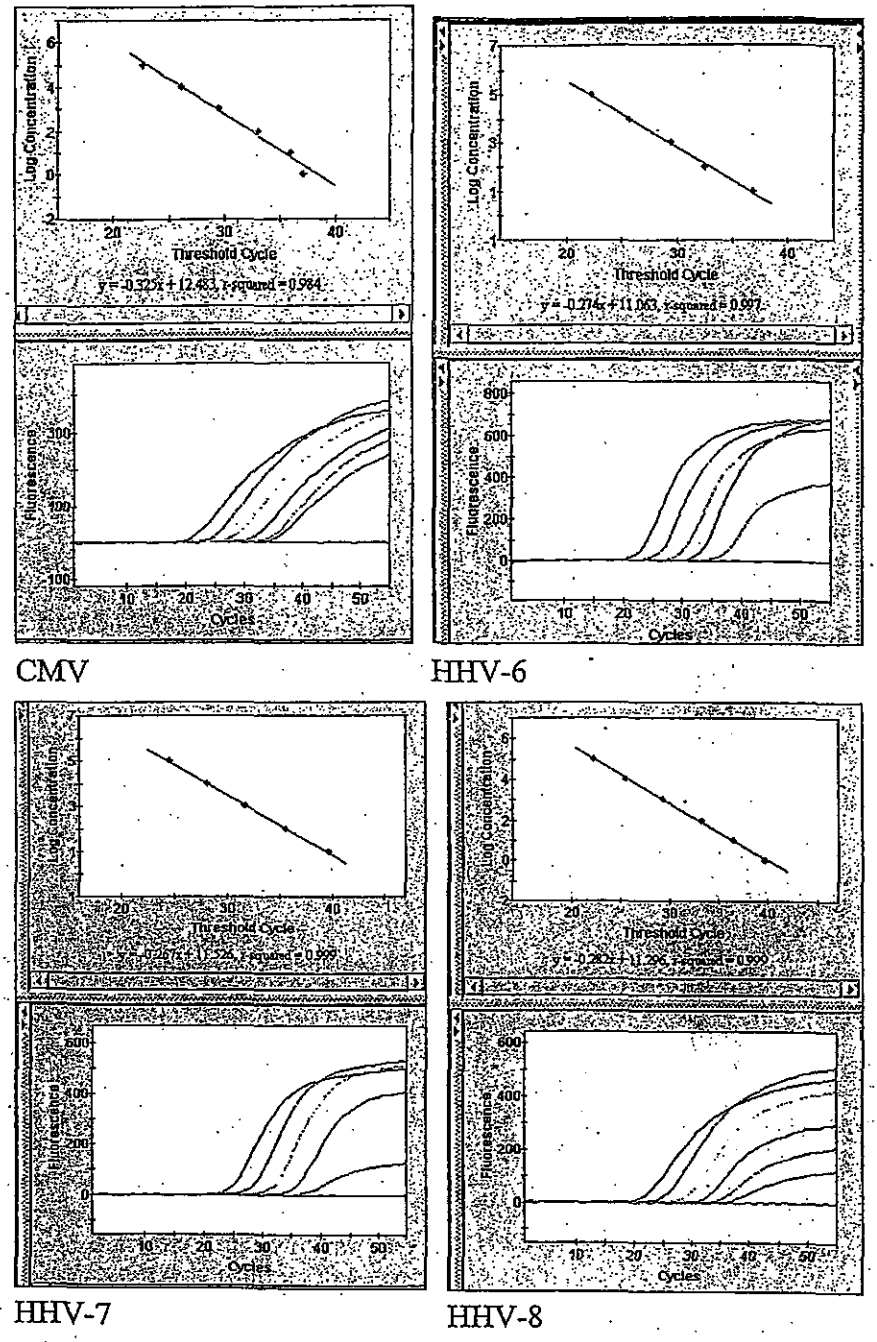


**Fig. 1.** *Continued*

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 $2 \times 10^6$ 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,