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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

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。

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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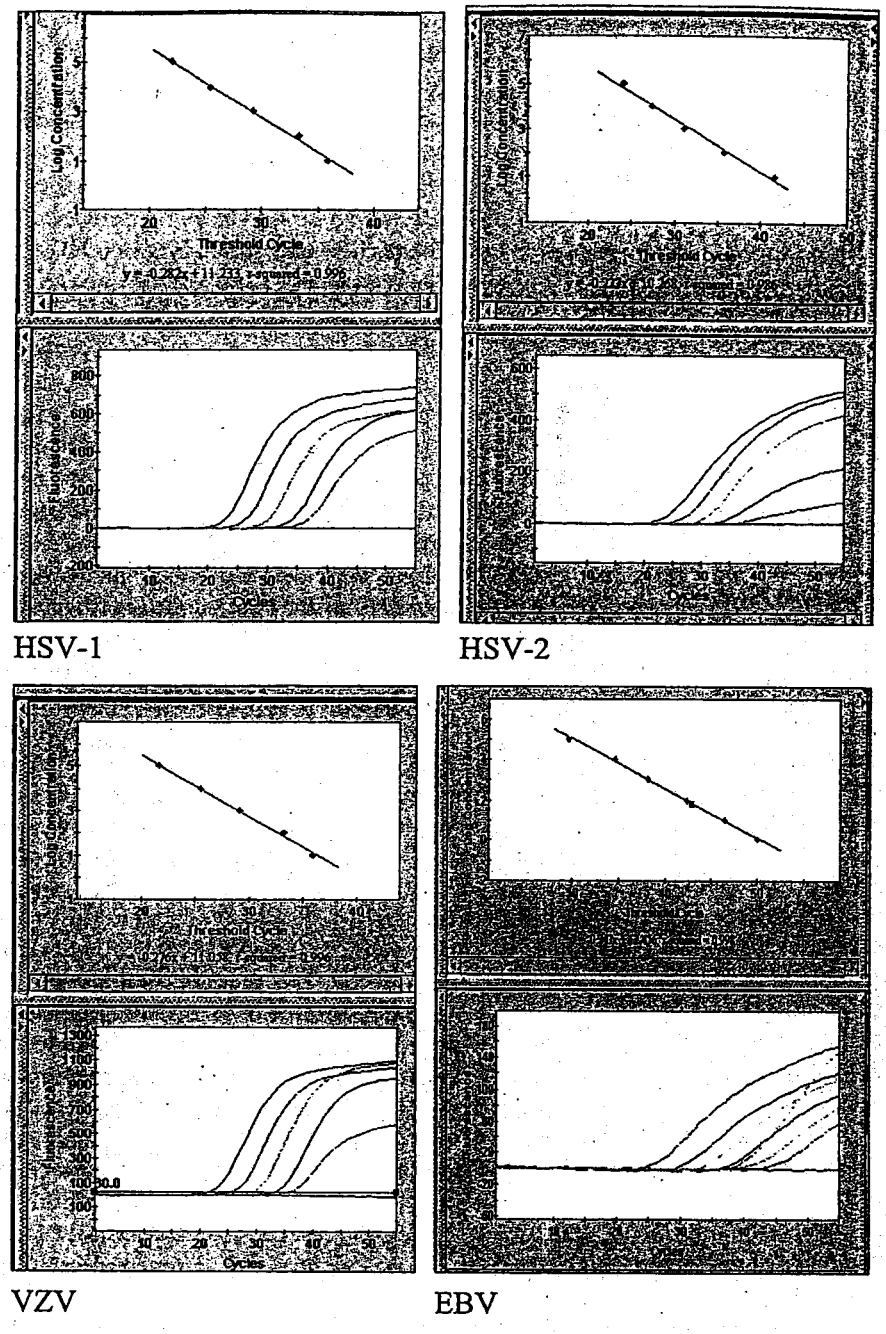

Fig. 1. Real-time PCR standard curves. The top panel displays the linear relationship between log concentration of viral DNA and PCR cycle. The bottom panel demonstrates the relationship between fluorescence signal intensity and PCR cycle. The curves from left to right in the lower panel represent serial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

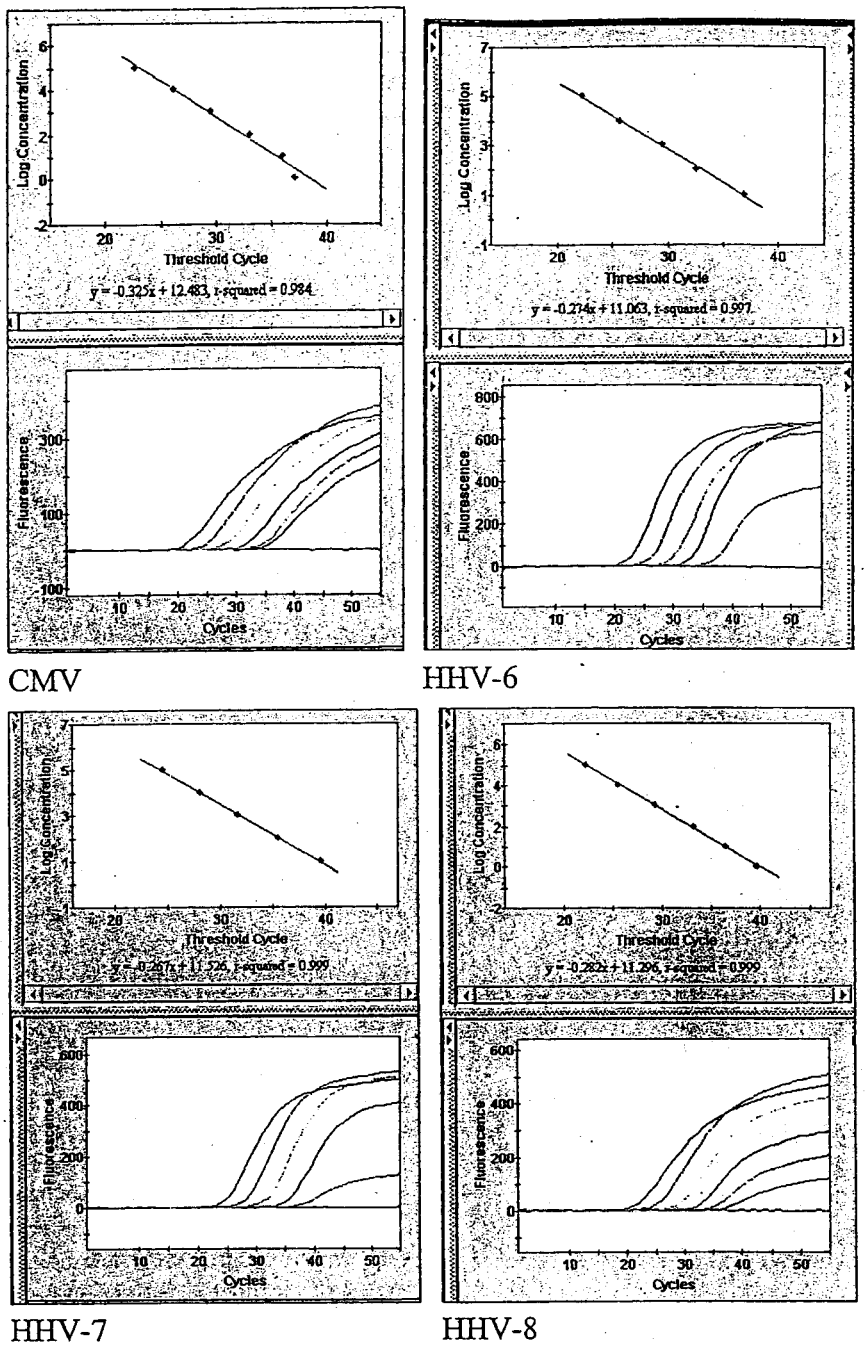

Fig. 1. *Continued*

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.

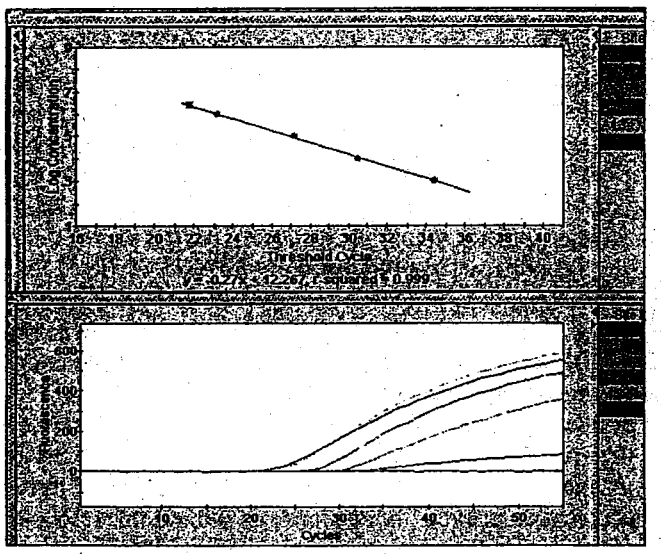

Fig. 2. Real-time PCR standard curve for HHV-6 high-viral-load samples. PCR positivity of extremely high HHV-6 viral loads (>10^6 copies/reaction) was seen only after more than 20 cycles of single-step PCR and yields a highly linear log standard curve with a range of 10^5 to 2.45×10^6 virus copies per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

ACKNOWLEDGMENT

The authors thank Ms Linda Beachey (UTMB Hematopathology) for her expert secretarial assistance.

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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
(This link will take you to the article abstract.)

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番号：2

別紙様式第２－１

医薬品　研究報告　調査報告書

識別番号・報告回数			報告日	第一報入手日 平成 20 年 10 月 20 日	新医薬品等の区分 該当なし	機構処理欄
一般的名称	ﾃｸﾈﾁｳﾑ人血清ｱﾙﾌﾞﾐﾝ (99mTc)		研究報告の公表状況	WHO ／ EPR ／ Disease Outbreak News,13 October 2008	公表国 ザンビア、南アフリカ	
販売名（企業名）	テクネアルブミンキット（富士フイルムＲＩファーマ株式会社）					
研究報告の概要	要約：南アフリカ共和国、ザンビア共和国におけるアレナウイルス科の新型ウイルス： 南アフリカ共和国とザンビア共和国で原因不明の疾患で死亡した３名はアレナウイルス科のウイルスによるものであることが検査の結果判明した。					使用上の注意記載状況・その他参考事項等 特になし
報告企業の意見			今後の対応			
アレナウイルス科の新ウイルスにより、３名が死亡したとの報告であり、また、初発症例はおそらくげっ歯類からの感染であるが、その後の症例は、ヒトーヒト感染による感染であること、及び新規かつ重大な感染症の研究報告と判断しております。また詳細は不明であるが、ヒト血液を原料とする血漿分画製剤とは直接関係するものではなく、現時点では、とくに措置等は必要ないと判断する。			現在、アレナウイルスの型を含めて、さらに詳細な調査が進められているところであり、今後とも追加・関連情報に注目し、情報入手次第、必要に応じて、再評価を行う予定とする。			

MedDRA/J Version(11.1)

World Health Organization

New virus from Arenaviridae family in South Africa and Zambia - Update

13 October 2008 -- The results of tests conducted at the Special Pathogens Unit, National Institute for Communicable Diseases (NICD) of the National Health Laboratory Service in Johannesburg, and at the Special Pathogens and Infectious Disease Pathology branches of the Centers for Disease Control in Atlanta, USA, provide preliminary evidence that the causative agent of the disease which has resulted in the recent deaths of 3 people from Zambia and South Africa, is a virus from the Arenaviridae family.

Analysis continues at the NICD and CDC in order to characterize this virus more fully. CDC and NICD are technical partners in the Global Outbreak Alert and Response Network (GOARN).

Meanwhile, a new case has been confirmed by PCR in South Africa. A nurse who had close contact with an earlier case has become ill, and has been admitted to hospital. Contacts have been identified and are being followed-up.

WHO and its GOARN partners continue to support the Ministries of Health of the two countries in various facets of the outbreak investigation, including laboratory diagnosis, investigations, active case finding and follow-up of contacts.

医薬品　研究報告　調査報告書

<table>
<tr><td>識別番号・報告回数</td><td colspan="2"></td><td>報告日</td><td>第一報入手日
2008年9月1日</td><td>新医薬品等の区分
該当なし</td><td rowspan="3">総合機構処理欄</td></tr>
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<tr><td>研究報告の概要</td><td colspan="5">問題点：インド東部において、原因不明のウイルス感染を原因とする急性脳炎症候群により、この数週間におよそ800人の患者が発生し150人が死亡している。

インド東部のウッタルプラデシ（Uttar Pradesh）州で小児を死亡させている原因不明のウイルスは、インド保健省の専門家らにより「acute encephalitis syndrome；急性脳炎症候群」（PT；感染性脳炎）と診断された。ウッタルプラデシ州の13の地区では、数週間におよそ800人の患者が発生し150人が死亡したと報告され、その数は増加すると見られている。
血液検査で、毎年この地方で大きな流行が発生する日本脳炎が陽性となったのは5%以下である。
通常、感染流行中は、15～20%以上の検体が日本脳炎陽性となる。しかし、2008年は低率であることが混乱を生じている。
2008年、ウッタルプラデシ州27地区の4000万人がJEワクチン接種を受けたが、なぜ発熱が抑えられなかったのかに専門家らはとまどっている。中国製ワクチンSA 14-14-2には問題はなく、WHOにより日本脳炎に対する防御作用が確認されている、と保健省当局者は述べた。
日本脳炎陽性が5%以下であることから、日本脳炎と、2006年以来脳炎の原因となっている水系感染のエンテロウイルスとの混合感染について調査が行われている。</td><td>使用上の注意記載状況・
その他参考事項等

記載なし</td></tr>
<tr><td colspan="3">報告企業の意見</td><td colspan="3">今後の対応</td></tr>
<tr><td colspan="3">別紙のとおり</td><td colspan="3">今後とも関連情報の収集に努め、本剤の安全性の確保を図っていきたい。</td></tr>
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MedDRA/J ver.11.0

別紙

一般的名称	①人血清アルブミン、②人血清アルブミン、③人血清アルブミン*、④人免役グロブリン、⑤乾燥ペプシン処理人免疫グロブリン、⑥乾燥スルホ化人免疫グロブリン、⑦乾燥スルホ化人免疫グロブリン*、⑧乾燥濃縮人活性化プロテインC、⑨乾燥濃縮人血液凝固第Ⅷ因子、⑩乾燥濃縮人血液凝固第Ⅸ因子、⑪乾燥抗破傷風人免疫グロブリン、⑫抗 HBs 人免疫グロブリン、⑬トロンビン、⑭フィブリノゲン加第ⅩⅢ因子、⑮乾燥濃縮人アンチトロンビンⅢ、⑯ヒスタミン加人免疫グロブリン製剤、⑰人血清アルブミン*、⑱人血清アルブミン*、⑲乾燥ペプシン処理人免役グロブリン*、⑳乾燥人血液凝固第Ⅸ因子複合体*、㉑乾燥濃縮人アンチトロンビンⅢ
販売名(企業名)	①献血アルブミン 20 “化血研”、②献血アルブミン 25 “化血研”、③人血清アルブミン “化血研” *、④ “化血研” ガンマーグロブリン、⑤献血静注グロブリン “化血研”、⑥献血ベニロンーⅠ、⑦ベニロン*、⑧注射用アナクトC2,500 単位、⑨コンファクトF、⑩ノバクトM、⑪テタノセーラ、⑫ヘパトセーラ、⑬トロンビン “化血研”、⑭ボルヒール、⑮アンスロビンP、⑯ヒスタグロビン、⑰アルブミン 20%化血研*、⑱アルブミン 5%化血研*、⑲静注グロブリン*、⑳ノバクトF*、㉑アンスロビンP1500 注射用
報告企業の意見	本感染症については、情報入手時点で病原因子は特定されていない。病原因子が細菌類であれば本剤の製造工程中の「無菌ろ過工程」および、細菌よりも小さいウイルスの除去を目的とした平均孔径 19nm 以下の「ウイルス除去膜ろ過工程」により除去されるものと考えられる。また、病原因子がウイルスであれば、「血漿分画製剤のウイルスに対する安全性確保に関するガイドライン（医薬発第 1047 号、平成 11 年 8 月 30 日)」に従ったウイルスプロセスバリデーションの結果から、病原因子は本剤の製造工程において除去・不活化されることが検証されている。以上のように、病原因子が細菌類あるいは既知のウイルスであれば、今回の感染症に対して本剤は一定の安全性を確保していると考える。また、未知のウイルスであっても、既存のウイルス除去・不活化工程の効果が期待される。 現時点で、感染症の流行はインド国内のみで当該生物由来成分の原産国とは離れているため、本剤への直接の影響はなく、緊急の安全対策の必要性もないと考えられるが、感染症は短期間に爆発的に増加することがあるため、今後とも関連情報の収集に努め、本剤の安全性の確保を図っていきたい。

*現在製造を行っていない

Archive Number 20080828.2697
Published Date 28-AUG-2008
Subject PRO/EDR> Undiagnosed fatal illness - India (04): (UP)

UNDIAGNOSED FATAL ILLNESS - INDIA (04): (UTTAR PRADESH)
****
A ProMED-mail post
<http://www.promedmail.org>
ProMED-mail is a program of the
International Society for Infectious Diseases
<http://www.isid.org>

[1]
Date: Tue 26 Aug 2008
Source: The Hindustan Times, online [edited]
<http://www.hindustantimes.com/StoryPage/StoryPage.aspx?sectionName=&id=3825990

The mystery virus striking children dead in eastern Uttar Pradesh (UP) has been diagnosed as "acute encephalitis syndrome" by Union Health Ministry experts. Simply put, they do not know what is causing the acute brain fever.

Within weeks, about 800 cases and 150 deaths were reported from 13 districts in UP, and experts predict that the numbers could rise.

"Less than 5 per cent blood and serum samples have tested positive for Japanese encephalitis (JE), which has seen major outbreaks in the region each year," said Dr Shiv Lal, director of the National Institute of Communicable Diseases.

"Usually, at least 15-20 per cent samples test positive for JE during an outbreak, but the low positivity is causing confusion this year [2008]. With 4 crore [40 million] children in 27 districts in UP being vaccinated against JE this year, experts wonder why the fever refuses to go away. There is no problem with the Chinese vaccine SA 14-14-2," said a health ministry official. The virus, approved by World Health Organization, protects against JE. "We suspect some children could have missed the vaccination drive." All the hospitalised children have reported symptoms of acute encephalitis.

"Since less than 5 per cent have tested positive for JE, we are investigating whether the outbreak is a combination of JE and water-borne enterovirus that caused the disease in 2006," said Dr Lal. The Centre is sending a 4-member team comprising a microbiologist, a pediatrician, an entomologist, and an epidemiologist to Lucknow and Gorakhpur to track the outbreak and collect blood and serum samples from hospitalisd children for viral culture.

"Apart from rapid tests for JE done using kits developed by Pune's National Institute of Virology, we will do virus culture to track the elusive cause of the current outbreak," said Dr Lal, adding that the result could be expected within 2 or 3 days of collection of the samples.

[byline: Sanchita Sharm]

--
communicated by:
ProMED-mail rapporteur Mary Marshall

******
[2]
Date: Wed 27 Aug 2008
From: T Jacob John <vlr_tjjohn@sancharnet.in>

Although the details are skimpy, age distribution and clinical description lacking, yet the available information can be used to propose a provisional diagnosis to be investigated. Heavy rainfall and flooding, febrile illness resembling malaria, and relatively large numbers of death does remind one of leptospirosis. Immediate serological testing for this disease is warranted.

Similar episodes in Orissa and Mumbai a few years ago (all the 3 features above fitted) turned out to be leptospirosis. In Orissa it was for the first time (at least recognized), while in Mumbai the presence of leptospirosis was already known. To add, there is no shortcut to detailed clinical description and elementary epidemiological investigation of cases based on specific diagnostic criteria of the outbreak disease, and exploration of risk factors (to look for transmission pathways). Instead of doing what one can do locally, the complete dependence on experts from elsewhere is not good.

--
Dr T Jacob John
Christian Medical College
Vellore
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[Japanese encephalitis virus infection is an unlikely explanation, but still under investigation. - Mod.CP

ProMED-mail thanks Dr John for his comments and looks forward to more information about this outbreak. - Mod.LL]

[see also:
Undiagnosed fatal illness - India (03): (UP) RFI 20080826.2666
Undiagnosed fatal illness - India (02): (UP) RFI 20080811.2478
Undiagnosed fatal illness - India (Uttar Pradesh): RFI 20080331.1194
2007

Japanese encephalitis - India (02) (Uttar Pradesh) 20071026.3486
Undiagnosed viral disease - India (02): (Uttar Pradesh) 20071026.3485
Undiagnosed viral disease - India: (Uttar Pradesh) 20071022.3440
Japanese encephalitis - India (Uttar Pradesh) 20070930.3233
2006

Japanese encephalitis - India (Uttar Pradesh) (03): vaccine safety 20061222.3583
Leptospirosis - India (Gujarat): not hantavirus 20060831.2476
Leptospirosis - India (Maharashtra) 20060726.2058
Leptospirosis - India (Kerala) 20060609.1612
Leptospirosis - India (Karnataka) 20060123.0226
2005

Undiagnosed deaths - India (Uttar Pradesh) (02) 20051115.3342
Undiagnosed deaths - India (Uttar Pradesh): RFI 20051113.3322
Leptospirosis - India (Maharashtra) 20050811.2348
2004

Leptospirosis - India (South Gujarat) (02) 20040908.2509
Leptospirosis - India (South Gujarat) 20040902.2441
Leptospirosis - India (Tamil Nadu) 20040226.0602]

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研究報告の概要

○ラテンアメリカおよびカリブ諸国のウエストナイルウイルスの活動性
目的:ウエストナイルウイルス(WNV)は、2001年に初めてカリブ海地域で検出されて以来、当地で急速に広がった。アメリカ大陸熱帯地域におけるWNV伝播の最近の知見について要約する。
方法:発表された文献のレビューを行い、主要な公衆衛生担当者に意見を求め、未発表データを入手した。
結果:WNV感染症は、ヒトでは2001年に初めてケイマン諸島およびフロリダキーの住民に発症し、2002年早期にジャマイカの健常な鳥類検体に初めて認められた。2002年のWNV感染症の血清学所見は、グアドループ、ドミニカ共和国と東部メキシコでウマ、ニワトリおよび野生鳥類に検出された。2003年には、WNVはメキシコおよび中央アメリカ北部で蔓延し、血清学的エビデンスはバハマ、プエルトリコとキューバで検出された。2004年9月～10月には、コロンビアとトリニダードで南米生態系におけるWNV活動の最初の血清学的エビデンスが表面化し、当地では家畜のWNV中和抗体保有率が高かった。
結論:ラテンアメリカおよびカリブ海地域において、ウマ、ヒトおよびトリでの疾患報告が少ないことは不可解である。熱帯生態系での疾患の低減について、ウイルスの減弱化あるいは他の可能性を検討するため、分離株が必要である。

使用上の注意記載状況・その他参考事項等

合成血-LR「日赤」
照射合成血-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

報告企業の意見	今後の対応
ラテンアメリカおよびカリブ海地域の動物や鳥類において、ウエストナイルウイルスの抗体陽性率は高くなっているが疾患報告は少ない。この不可解な熱帯生態系での疾患の低減について、原因を検討するため分離株が必要であるとの報告である。	日本赤十字社では、輸血感染症対策として問診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、ウエストナイルウイルス感染の発生に備え、緊急対応の準備を進めている。今後も引き続き情報の収集に努める。

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Informe especial / Special report

West Nile virus activity in Latin America and the Caribbean

Nicholas Komar[1] *and Gary G. Clark*[2]

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ABSTRACT

Objectives. *West Nile virus* (Flavivirus: Flaviviridae; *WNV) has spread rapidly throughout the Caribbean Basin since its initial detection there in 2001. This report summarizes our current knowledge of WNV transmission in tropical America.*

Methods. *We reviewed the published literature and consulted with key public health officials to obtain unpublished data.*

Results. *West Nile virus infections first appeared in human residents of the Cayman Islands and the Florida Keys in 2001, and in apparently healthy Jamaican birds sampled early in 2002. Serologic evidence of WNV infection in 2002 was detected in horses, chickens and resident free-ranging birds in Guadeloupe, the Dominican Republic, and eastern Mexico. In 2003, WNV spread in Mexico and northern Central America, and serologic evidence was detected in the Bahamas, Puerto Rico and Cuba. In 2004, the first serologic evidence of WNV activity in South American ecosystems surfaced in September-October in Colombia and Trinidad, where domestic animals circulated WNV-neutralizing antibodies.*

Conclusions. *The sparse reports of equine, human and avian disease in Latin America and the Caribbean is puzzling. Isolates are needed to evaluate viral attenuation or other possible explanations for reduced disease burden in tropical ecosystems.*

Key words West Nile virus; Latin America; Caribbean region; arboviruses; population surveillance; flavivirus.

INTRODUCTION

Since West Nile virus (*Flavivirus: Flaviviridae*; WNV) first appeared in the Western Hemisphere in New York in 1999, it has spread rapidly across the North American continent, causing large numbers of human cases with neurologic disease and death, and even greater amounts of milder disease characterized principally by fever and rash. Horses and hundreds of species of birds also fell victim to this emerging virus (1). West Nile virus spread southward into the Caribbean Basin and Latin America as well, where its public health impact remains poorly understood and surveillance systems are unprepared to track its spread. The virus was first detected in 2001, in Jamaica and the Cayman Islands. In 2005 WNV activity was reported from many locations in the Caribbean Basin, Mexico, Central America and the northern rim of South America (Figure 1). In order to package our current knowledge of WNV activity and surveillance results from various locations within tropical America, we reviewed published reports and some unpublished data available from public health officials, and provide a summary below. We also comment on the significance of the surveillance findings and on the potential public health threat of WNV in tropical America.

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METHODS

We reviewed peer-reviewed publications and government reports and consulted with key public health officials within Caribbean Basin countries to obtain unpublished data.

RESULTS

West Nile virus detected in 2001

In the State of Florida (United States of America), Blackmore et al. described surveillance findings for WNV in two epidemic foci in 2001—a northern focus and a southern focus (2). The northern focus was characterized by humid temperate forests typical of the southeastern United States but unlike tropical ecosystems in Latin America. The first evidence for WNV activity here was a dead American Crow (*Corvus brachyrhynchos*) in June, 2001. Nine human cases of West Nile neurologic disease (WNND) were reported between July and October. Entomologic investigations near case residences in July detected WNV in three species of *Culex* (*Culex*) mosquitoes: *Culex quinquefasciatus*, *C. nigripalpus* and *C. salinarius* (3, 4). The first two of these species are common further south in the Caribbean Basin.

The southern epidemic focus in Florida was more typical of Caribbean Island ecology and occurred in the Florida Keys. A human case of WNND with onset in July, 2001, represented the earliest indication of WNV activity there. Two more human cases were reported with onsets in August and September. West Nile virus was isolated from dead corvids (e.g., Fish Crow, *Corvus ossifragus*) and *Streptopelia* doves (probably *Streptopelia decaocto*, Eurasian Collared-Dove, an introduced species that is also abundant in the Bahamas). Entomologic investigations were carried out throughout the Keys during the last quarter of 2001 (5). Infection rates were highest in *Anopheles atropos* (3 of 410), *Deinocerites cancer* (2 of 845) and *Ochlerotatus taeniorhynchus* (2 of 9288). This last species is a ferocious human biter, and abundant in coastal locations throughout the Caribbean Basin. About 20 000 other mosquitoes tested negative.

Follow-up mosquito surveillance studies in the Florida Keys in the following two years yielded no WNV in more than 30 000 mosquitoes tested in 2002, but the virus was detected in 10 pools representing 53 673 mosquitoes in 2003 (6). In 2003, infections were detected from May–September. Infected species included *C. quinquefasciatus* (minimum infection rate 1.7 per 1 000), *C. nigripalpus* (0.9), *O. taeniorhynchus* (0.9), *O. condolescens* (0.6) and *C. erraticus* or *declarator* (0.6). No infections were detected in either *A. atropos* or *D. cancer* even though more than 5 000 of each species were tested. These findings suggest that either WNV became endemic in the Florida Keys but dropped below levels of detection in 2002, or that multiple, temporally dispersed introductions occurred, resulting in transmission activity in both 2001 and 2003.

Although the circumstances of WNV introduction into the Florida Keys are unknown, the likely explanation is that migrating birds served as dispersal hosts, seeding the virus into potential transmission foci during their southward migration in the fall of 2000. By late 2000, WNV activity was reported as far south as North Carolina in the continental United States (7). The virus had probably spread even further south at undetectable levels, to be amplified by resident birds and *Culex* mosquitoes during the warmer spring and early summer months of 2001. While migrating birds are a convenient explanation of WNV dispersal, other possible means of dispersion exist, such as infected mosquitoes that are accidentally transported via surface transportation or airplanes.

South of the Florida Keys, a human WNND case with no history of international travel was reported with onset on August 2, 2001, from tiny Cayman Brac (area 14 square miles [36 square kilometers], population 1 200), in the Cayman Islands, south of Cuba (8). Assuming an incubation period of 2–15 days in people, this infection probably occurred in late July, about the same time that the first human case was infected in the Florida Keys. However, the laboratory diagnosis of this case was not announced until October 15, 2001. Laboratory tests were positive for anti-WNV IgM (indicating recent infection) and a 90% plaque-reduction neutralizing antibody titer ($PRNT_{90}$) of 1:1280, compared with a $PRNT_{90}$ of 1:80 and <1:10 for St. Louis encephalitis virus (SLEV) and Dengue-2 virus, respectively (CDC, unpublished data).

More data supporting WNV transmission activity in the Caribbean Basin in 2001 came from Jamaica, where a Smithsonian Institution-New York State Health Department research team reported 17 seropositive resident birds of 348 collected in 3 of 4 study sites, all on the western side of the island (9). The samples were collected in the first three months of 2002 but probably reflected transmission that had occurred months earlier in 2001. Seropositive bird species included *Turdus aurantius* (n = 4), *Myiopagis cotta* (2), *Coereba flaveola* (2), *Tiaris bicolor* (2), and one each of seven other species. Seropositivity was determined by comparing $PRNT_{90}$ titers for WNV, SLEV and Ilheus virus, a South American flavivirus that is genetically closely related to SLEV, but not in the same antigenic complex as SLEV and WNV (10). All 17 WNV-positive samples were at least four-fold greater in WNV titer than other flavivirus titers. Three samples were positive for SLEV-neutralizing antibodies, which has been previously isolated in Jamaica (11). No samples were positive for Ilheus virus, but five additional samples had similar titers for both SLEV and WNV, and these were classified as undetermined flavivirus infections. The 2001 WNV activity in Jamaica and the Cayman Islands was most likely the result of the same introduction mechanism as postulated for extreme southern Florida: southward dispersal of the virus below limits of detection via migrating birds late in 2000.

Operating under the premise that birds would carry WNV along migration routes, efforts were initiated to detect WNV activity on the southern side

of the Gulf of Mexico, where millions of neotropical migratory birds make landfall each year and spend the winter months. Beginning in 2000, a joint effort by the Universidad Autónoma de Yucatán and Colorado State University blood-sampled and tested migratory and resident birds in Yucatán State, Mexico. The following year, the Smithsonian Institution also began sampling birds on the Yucatán Peninsula. Further south in the Lacandón Forest of Chiapas State, a joint federal Mexico-United States study evaluated blood from about 200 resident domestic animals sampled in July of 2001. From these, a single seropositive cow (*Bos* sp.) with a $PRNT_{90}$ for WNV of 1:80 and a $PRNT_{90}$ for SLEV of 1:20, was considered a probable case of WNV infection (12). However, these authors cautioned against concluding that WNV had reached southern Mexico. They reasoned that a major range extension should be confirmed by a second detection of infection. Also, no evidence of WNV transmission had been detected at that time in the nearby Yucatán Peninsula (9, 13). The Chiapas study demonstrated serologic evidence for infections due to uncharacterized flaviviruses which could have resulted in cross-reaction with WNV. Secondary flavivirus infections are notorious for causing elevated heterologous flavivirus titers (14).

FIGURE 1. Countries of Latin America and the Caribbean with reported activity for West Nile virus (in black) between 2001 and 2004, including Mexico, Belize, Guatemala, El Salvador, Cuba, Bahamas, Cayman Islands, Jamaica, Dominican Republic, Puerto Rico (United States), Guadeloupe (French West Indies), Trinidad and Tobago, and Colombia

Spread of West Nile virus 2002–2004

In 2002, WNV continued to spread in the Caribbean Basin. Guadeloupe (French West Indies) reported numerous subclinical infections in horses and chickens, determined serologically by neutralization (15). In July 2002, 10.4% of the healthy horses in four locations were positive and by January 2003, 61.6% had become positive in these locations. The absence of reported neurologic disease in these horses is mysterious. Subsequent surveillance in 2003 and 2004 failed to detect any transmission (16).

In the Dominican Republic on the Greater Antillean island of Hispaniola, a University of Kansas study team sampled blood and tissues from resident birds captured in November, 2002, for museum collections (17). Five birds of 33 (15.2%) from the Parque Nacional Los Haitises on the northeast coast tested positive for WNV antibodies by neutralization and a specific inhibition-ELISA test. A follow-up study in March, 2003, yielded 12 more WNV-seropositive birds of 58 (20.7%) at the Parque Nacional Monte Cristi in northwest Dominican Republic, along the border with Haiti (18). Positive Dominican bird species included *Phaenicophilus palmarum* (n = 4), two each of *Ploceus cucullatus, Saurothera longirostris, Loxigilla violacea* and *Turdus plumbeus*, and one each of five other species.

Evidence of WNV infection was confirmed in Mexico as of July, 2002. Seropositive horses were reported from six states (Chihuahua, Coahuila, Tamaulipas, Veracruz, Tabasco and Yucatán) (19–21). Seropositive birds were rare and were first detected in the early winter months of 2003 (13, 22).

Mexican authorities began widespread serosurveys in horses and birds in 2003 and found many seropositive horses in 22 states (J. Mendez, personal communication, 4 Feb 2004), with no human cases in 2003 and six human cases (three with encephalitis) in northern Mexico in 2004 (23). The first Mexican isolate came from a dead captive common raven (*Corvus corax*) in Tabasco State (southeast Mexico) in May, 2003 (21). Additional isolates from dead birds were obtained in northwest Mexico later in 2003 and 2004. Phylogenetic analysis of the prM-E region of the WNV genome isolated from the raven in Tabasco linked it to central United States strains from 2002, but revealed slightly greater genetic variation than previous

reports for North American WNV strains (21). Two of the 9 nucleotide mutations resulted in amino acid changes, and one of these altered a glycosylation site within the envelope (E) protein. Virulence testing of plaque-purified subcultures of this isolate revealed variants with reduced virulence in mice (24). Similar observations had been made with a Texas 2002 isolate (25).

The widespread WNV seropositivity among horses observed in Mexico in 2003 was also present in the Central American republics of El Salvador and Guatemala (26, M.E. Morales-Betoulle et al., manuscript in preparation). However, anecdotal reports of fatal or life-threatening neurologic disease in Mexican and Central American horses have rarely been confirmed as due to WNV. One encephalitic horse diagnosed with WNV infection was reported from Belize, with onset October 31, 2003. Interestingly, 2 000 birds sampled in Belize earlier in 2003 and another 2 000 in 2002 all tested negative for WNV antibodies (27).

West Nile virus activity continued in the eastern Caribbean region in 2003. In the Bahamas, a human case of WNND was diagnosed with onset in July, 2003 (28). In early 2004, two seropositive *Turdus plumbeus* (of 734 birds sampled) were detected in Guantanamo Bay Naval Base at the eastern point of Cuba, and in eastern Puerto Rico, one *Coereba flaveola* (of 1 200 birds sampled) was seropositive, probably reflecting transmission in 2003 (29). Three neutralizing antibody- and IgM-positive, healthy horses were also reported in eastern Puerto Rico in May, 2004, and two others were found in central Puerto Rico in July, 2004 (A. Diaz et al., manuscript in preparation). Mosquitoes collected from the locations where seropositive horses resided tested negative for WNV infection. Four seropositive horses from the Havana region and three human WNND cases in central Cuba were announced in January, 2005 (G. Kouri, personal communication, 2 February 2005), reflecting transmission in 2004.

In the fall of 2004, 8 resident unvaccinated horses (of 200 sampled) and 2 domestic Muscovy ducks (of 40 resident birds sampled) were seropositive for WNV in Trinidad (28; R. Salas, personal communication, 17 November 2005), and 12 seropositive equines (of 130 sampled) were reported in northern Colombia (30). These reports mark the first evidence of WNV activity in South American ecosystems (the island of Trinidad is located within sight of the South American mainland off the coast of Venezuela). Efforts to detect WNV-specific antibodies in resident and migrant birds in Brazil in 2002 and 2003 were unsuccessful (31). With the incursion of WNV into northern South America in 2004, it becomes the only zoonotic flavivirus to have been identified in six continents.

DISCUSSION

The failure of efforts to isolate the virus or detect genomic RNA from WNV in Latin America and the Caribbean (with a few exceptions in Mexico) is perplexing and underscores the concern that serologic evidence for WNV activity is at best indirect. Flaviviruses are notorious for their close antigenic relationships and serologic cross-reactivity (10). In spite of strong serologic evidence from cross-neutralization testing against known flaviviruses from the region, the possibility of misdiagnosis due to cross-reaction with an as yet unrecognized "WN-like" virus still exists. In fact, some of the serologic results classified as due to "undifferentiated flavivirus infection" can best be explained by the existence of such a virus. The recent discovery of two strains of WN-like virus in central Europe lends credence to this concern (32). These two WN-like viruses were both identified serologically as WNV, but genetically they are equidistant from both currently recognized WNV lineages and each other and may represent newly discovered WNV lineages or new WN-like flaviviruses.

Another concern is the strong emphasis placed by several research groups on serologic surveillance of migratory birds (9, 13, 22, 29, 31). These studies consume large quantities of valuable resources, yet are unlikely to provide significant results. Given the recent intense transmission of WNV during the summers in temperate North America, the capture of WN-seropositive avian survivors either during migration or on the wintering grounds is to be expected because many of these birds normally migrate to neotropical winter territories where they probably continue to circulate antibodies derived from a WNV infection acquired on their North American breeding grounds. Some studies claim that seropositive migrants are evidence that birds could carry WNV long distances. Unfortunately, although plausible, this conclusion is not valid for two reasons. First, the possibility that WN-seropositive migratory birds were in fact infected locally cannot be disproved. Second, long-distance migration by a healthy, antibody-circulating bird does not indicate that a viremic bird could make the same long-distance flight. More data are needed to support such a hypothesis. However, the observation of infectious WNV at high titers in tissues of convalescent migratory birds (e.g., Killdeer, *Charadrius vociferus*) more than one week post-infection and the demonstration of oral infection in raptors would suggest that recently infected birds that recover from viremia, migrate, and then fall prey to a raptor may still introduce WNV into new distant ecosystems if the raptor becomes infected and circulates sufficient virus in its blood to infect mosquitoes (33).

The most pressing concern regarding the reports of WNV in Latin America and the Caribbean is the absence of data on the disease burden in people, horses or birds. Widespread resistance to virulent strains of WNV in Latin American and Caribbean vertebrates (including people) seems highly unlikely. However, the selection of resistant WNV strains is plausible. If migrating birds are indeed the major mechanism for southward dispersal of WNV, then one could imagine a scenario in which birds infected with highly virulent strains become too sick to migrate, while birds infected with avirulent strains make the long flights

across seas and deserts successfully, spreading avirulent WNV to new transmission foci along their migratory routes. More research is needed to evaluate this hypothesis, but if proven, this bodes well for the future of WNV epidemics in North America, as the avirulent strain might be reintroduced continually from the south by returning migratory birds. South American arboviruses have in fact been isolated from northward-bound birds during the spring migration in Louisiana (34). This scenario may also explain the apparent low virulence for SLEV in birds and horses in North and South America. In fact, South American strains of SLEV are also less viremogenic in birds than are North American strains, and less virulent in mice (35). Whether an avirulent bird and horse strain of WNV will also be less virulent for humans remains to be seen.

Saint Louis encephalitis virus may be responsible for considerable cross-reaction to WNV in serologic tests of serum from Latin America. The virus is expected to cross-react in about 5% of primary WNV infections of birds (36). However, in secondary infections, the proportion of samples that cross-react by PRNT is probably much greater. Secondary flavivirus infections may explain the high rate of flavivirus antibody-positive serum samples in the Caribbean Basin countries that cannot be assigned to a specific infection (because of the presence of similar titers for multiple flaviviruses). Although rarely associated with disease in Latin America, SLEV infections are commonly reported. For example, in Chiapas, Mexico, 20 (10%) of 196 domestic animals (including three of five horses) were diagnosed as positive for SLEV-neutralizing antibodies by PRNT (12). The known range of distribution for SLEV was expanded through the efforts to detect WNV in the Caribbean Basin. For example, two SLE-seropositive birds reported in Puerto Rico provide the first evidence of SLEV activity from that Caribbean location (9, 29).

CONCLUSION AND RECOMMENDATIONS

Although WNV has yet to present a serious disease threat in Latin America and the Caribbean Basin, an outbreak may be pending. The first major outbreak (with >100 human cases of WNND) in the United States was delayed until 2002, three years after initial detection of the virus in 1999. Public health and veterinary authorities in Latin America and the Caribbean should remain vigilant for unusual clusters of severe disease cases. Dead birds (especially corvids) have been particularly useful for the early detection of WNV activity in North America (37). Corvids are less abundant in Latin America, and thus avian mortality may be less useful as a surveillance technique in this region (38). In countries where WNV has already been detected, surveillance efforts should be expanded. Surveillance guidelines for Latin American and Caribbean Basin countries are available (28, 39, 40).

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RESUMEN

La actividad del virus del Nilo occidental en América Latina y el Caribe

Objetivos. El virus del Nilo occidental (VNO, familia Flaviviridae, género *Flavivirus*) se ha propagado rápidamente por toda la cuenca del Caribe desde que se detectó por primera vez en 2001. En este informe se resumen nuestros conocimientos actuales acerca de la transmisión del VNO en zonas tropicales del continente americano.

Métodos. Revisamos todo lo que se ha publicado sobre el tema y consultamos a autoridades de salud clave para obtener datos inéditos.

Resultados. Las infecciones por el virus del Nilo occidental aparecieron por primera vez en seres humanos residentes de las Islas Caimán y de los Cayos de la Florida en 2001, y en pájaros de aspecto sano de los cuales se obtuvieron muestras a principios de 2002. En 2002 se encontraron pruebas serológicas de infección por el VNO en caballos, pollos y aves de corral no estabuladas oriundas de Guadalupe, la República Dominicana y la parte oriental de México. En 2003, el VNO se diseminó dentro de México y por la parte norte de Centroamérica y se encontraron pruebas serológicas en las Bahamas, Puerto Rico y Cuba. En 2004, las primeras pruebas serológicas de actividad vírica en ecosistemas sudamericanos se detectaron en septiembre y octubre en Colombia y Trinidad, donde se observaron anticuerpos neutralizantes contra el VNO en animales domésticos.

Conclusiones. Estos informes esporádicos de enfermedad equina, humana y aviar en América Latina y el Caribe son desconcertantes. Es necesario aislar las cepas para determinar si la atenuación del virus u otro factor explica la carga de enfermedad reducida en ecosistemas tropicales.

Palabras clave Virus del Nilo occidental, América Latina, región del Caribe, arbovirus, vigilancia de la población, flavivirus.

別紙様式第2-1

No. 19

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
				2008. 7. 11	該当なし	

一般的名称	(製造販売承認書に記載なし)	研究報告の公表状況		公表国
販売名(企業名)	合成血-LR「日赤」(日本赤十字社) 照射合成血-LR「日赤」(日本赤十字社)		ProMED 20080709.2092, 2008 Jul 09. 情報源:Turkish Daily News, 2008 Jul 9.	トルコ

研究報告の概要

○クリミア・コンゴ出血熱―トルコ

2008年7月7日、トルコのブルサ、チャナッカレ、サムスンの病院で3名の患者がダニ媒介性疾患のクリミア・コンゴ出血熱で死亡し、この2ヶ月間での死亡者数は37名になった。

保健省はダニに注意するよう呼びかける声明を発表した。ダニに咬まれた場合は決して手でつぶさずに、皮膚を保護し、医師にピンセットで注意深く取り除いてもらった後、ヨードで消毒することを推奨している。さらに、咬まれた人は10日間医学的観察を行い、発熱、頭痛、吐き気、嘔吐、下痢などの症状が現われた場合は、最寄りの病院を受診するよう、保健省の担当者は話している。

クリミア・コンゴ出血熱は主に動物に感染し、ヒツジや家畜に寄生するダニが、時折人にウイルスを感染させる。迅速に治療しないと出血によって死亡することもある。感染した人の血液や唾液を介して他の人にウイルスが伝播される可能性がある。感染地域はアフリカ、アジア、ヨーロッパの一部だが、近年トルコの気候が温暖になっていることから、ダニの数が増えてより多くの人が感染するようになっていると保健当局では話している。

報告企業の意見	今後の対応
2008年7月7日、トルコの病院で3名の患者がダニ媒介性疾患のクリミア・コンゴ出血熱で死亡し、この2ヶ月間での死亡者数は37名になったとの報告である。	日本赤十字社では、輸血感染症対策として問診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。今後も引き続き、新たな病原体による感染症の発生状況等に関する情報の収集に努める。

使用上の注意記載状況・その他参考事項等

合成血-LR「日赤」
照射合成血-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

19

Archive Number 20080709.2092
Published Date 09-JUL-2008
Subject PRO/AH/EDR> Crimean-Congo hem. fever - Turkey (11)

CRIMEAN-CONGO HEMORRHAGIC FEVER - TURKEY (11)
****
A ProMED-mail post
<http://www.promedmail.org>
ProMED-mail is a program of the
International Society for Infectious Diseases
<http://www.isid.org>

Date: Wed 9 Jul 2008
Source: Turkish Daily News, Dogan News Agency report [edited]
<http://www.turkishdailynews.com.tr/article.php?enewsid=109351>

On Mon 7 Jul 2008, 3 people were pronounced dead at hospitals in the provinces of Bursa, Canakkale, and Samsun, taking the death toll from tick bites to 37 in the past 2 months. According to the Dogan news agency, a resident of the western province of Bursa went camping 10 days ago and was bitten by a tick. He was hospitalised and diagnosed with the deadly Crimean-Congo hemorrhagic fever (CCHF), and moved to the intensive care unit.

In the western province of Canakkale, a man died in hospital after being treated for suspected CCHF infection. He had told relatives that he had seen a tick on his body. He was buried in a zinc casket with lime spread over the grave as a precaution. Another person had died from CCHF in the same province last month [June 2008].

Another man died from CCHF on Monday [7 Jul 2008] in the northern province of Samsun after he was bitten by a tick and removed it with his hand.

The Health Ministry also issued a statement to warn people against ticks. In case of a tick bite the skin should be covered with [an antiseptic]. The tick should be removed by doctors using tweezers with great care and iodine should be applied to the bite. Health Ministry officials said ticks should never be killed by hand.

Moreover, those people, touched by any tick, should be kept under medical observation for 10 days, and go to the nearest hospital if they have symptoms such as fever, headache, nausea, vomiting, or diarrhea, officials from the Health Ministry said.

CCHF mainly affects animals. Ticks, which live on sheep and cattle, can sometimes pass the virus to people. It is a [haemorrhagic] fever where patients can bleed to death if they are not treated quickly. Those infected can transmit the virus through their blood or saliva. The disease is endemic in parts of Africa, Asia, and Europe. Health authorities said a warmer climate, which Turkey has experienced in recent years, could mean a larger tick population that could in turn infect more people with the disease.

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Communicated by:
ProMED-mail Rapporteur A-Lan Banks

[The CCHF death toll in Turkey has risen from 33 on 4 Jul 2008 , when more than 550 cases were recorded, to the present 37.

The HealthMap/ProMED-mail interactive map of Turkey is available at
<http://healthmap.org/promed?v=39.1,35.2,5>,

and a map delineating the administrative provinces of Turkey can be accessed <http://www.mapsofworld.com/turkey/turkey-political-map.html>. - Mod.CP]

[see also:
Crimean-Congo hem. fever - Turkey (10): treatment 20080704.2038
Crimean-Congo hem. fever - Turkey (09) 20080622.1935
Crimean-Congo hem. fever - Turkey (08) 20080620.1917
Crimean-Congo hem. fever - Turkey (07) 20080616.1892
Crimean-Congo hem. fever - Turkey (06) 20080615.1888
Crimean-Congo hemorrhagic fever - Turkey (05) 20080612.1866
Crimean-Congo hem. fever - Turkey (04) 20080611.1838
Crimean-Congo hem. fever - Turkey (03) 20080522.1686
Crimean-Congo hem. fever - Turkey (02) 20080511.1610
Crimean-Congo hem. fever - Turkey 20080508.1567
2007

Crimean-Congo hemorrhagic fever - Turkey 20070610.1892
2006

Crimean Congo hemorrhagic fever - Turkey (05) 20060822.2359
Crimean-Congo hemorrhagic fever - Turkey (04): WHO 20060809.2230
Crimean-Congo hemorrhagic fever - Turkey (03): comment on tick removal 20060728.2082
Crimean-Congo hemorrhagic fever - Turkey (02) 20060722.2013
Crimean-Congo hemorrhagic fever - Turkey 20060705.1844]
.....cp/mj/dk

別紙様式第2-1

No. 8

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
				2008. 7. 11	該当なし	

一般的名称	(製造販売承認書に記載なし)	研究報告の公表状況	CDC, Salmonella Saintpaul Outbreak Notices. 2008 Jul 8; Available from: URL: http://www.cdc.gov/salmonella/saintpaul/archive/070808.html	公表国 米国
販売名(企業名)	合成血-LR「日赤」(日本赤十字社) 照射合成血-LR「日赤」(日本赤十字社)			

研究報告の概要

○サルモネラ・セントポール流行の調査

CDCは、関係機関と協力して、複数州でのサルモネラ菌血清型セントポールのアウトブレイクを調査している。当初の疫学調査では生のトマトの摂食が関連すると考えられたが、最近、レストランで食事をした患者で多くのクラスターが発生していることが判明した。このため、トマトと同時に摂食されることの多い生のハラペーニョやコリアンダーなども原因となった可能性があるが、現時点では、感染源をこのうちの一つに特定することはできない。

7月7日時点で、41の州、ワシントンD.C.、カナダで991名の患者が同じ遺伝子パターンのサルモネラ・セントポールに感染した。感染が特定された症例は、検査施設が州の衛生研究所にサルモネラ株を送って確認されたものである。患者のうち、情報が得られた711名は、4月10日～6月25日の間に発症し、このうち275名は6月に発症していた。患者の年齢は1歳～99歳で48%が女性だった。最も感染者の割合が高いのは20歳～29歳、最も低いのは10歳～19歳及び80歳以上の年代だった。感染に関連した死亡例が2例報告されている。2007年の4月～6月にこの型のサルモネラ・セントポールに感染した患者は6名しかいなかった。

公衆衛生当局は5月からアウトブレイクの調査を続けているが、患者が食べたものを正確に思い出し、食材を特定することが難しいため、調査は難航している。サルモネラに感染した場合、12～72時間で下痢、発熱、腹痛などの症状を発症し、4～7日間持続する。ほとんどの患者は自然に回復するが、小児、高齢者、免疫不全患者などでは重症化しやすい。FDAは感染源と見られるトマトの摂食を控え、サルサソースなどに使われる生のトマトにも注意するよう呼びかけている。

報告企業の意見	今後の対応
2008年7月7日時点で、米国の41の州、ワシントンD.C.、カナダで991名の患者がサルモネラ・セントポール株に感染したことが確認されたとの報告である。	日本赤十字社では、輸血による細菌感染予防対策として問診時に献血者の健康状態を確認し、発熱を伴う食中毒様の激しい下痢症状がある場合は1ヶ月間献血不適としている。また、全ての輸血用血液製剤について、平成19年1月より保存前白血球除去を実施している。今後も細菌やウイルスの検出や不活化する方策について情報の収集に努める。

使用上の注意記載状況・その他参考事項等

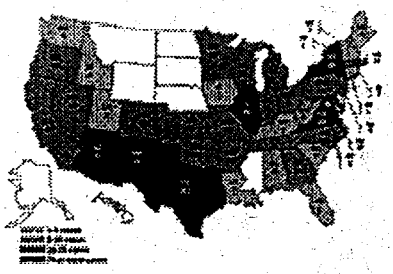
合成血-LR「日赤」
照射合成血-LR「日赤」

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

JRC2008T-046

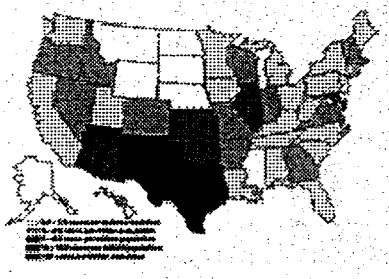
Investigation of Outbreak of Infections Caused by *Salmonella* Saintpaul

Cases infected with the outbreak strain of *Salmonella* Saintpaul, United States, by state, as of July 7, 2008 9pm EDT



Click map to view a larger image.

Incidence of cases of infection with the outbreak strain of *Salmonella* Saintpaul, United States, by state, as of July 7, 2008 9PM EDT



Click map to view a larger image.

Questions and Answers
Related to the Outbreak of *Salmonella* Saintpaul infections associated with tomatoes.

Update for July 8, 2008 - Case count information as of 9 pm EDT, July 7, 2008

Click Here for Advice to Consumers

CDC is collaborating with public health officials in many states, the Indian Health Service, and the U.S. Food and Drug Administration FDA) to investigate an ongoing multi-state outbreak of human *Salmonella* serotype Saintpaul infections. An initial epidemiologic nvestigation comparing foods eaten by ill and well persons identified consumption of raw tomatoes as strongly linked to illness. Recently, many clusters of illnesses have been identified in several states among persons who ate at restaurants. These clusters led us to broaden the investigation to be sure that it encompasses food items that are commonly consumed with tomatoes. Fresh tomatoes, fresh hot chili peppers such as jalapeños, and fresh cilantro are the lead hypotheses. However, at this point in the investigation, we can neither directly implicate one of these ingredients as the single source, nor discard any as a possible source.

Since April, 991 persons infected with *Salmonella* Saintpaul with the same genetic fingerprint have been identified in 41 states, the District of Columbia, and Canada. These were identified because clinical laboratories in all states send *Salmonella* strains from ill persons to their State public health laboratory for characterization. One new state, West Virginia, reported an ill person. The number of ill persons identified in each state is as follows: Alabama (2 persons), Arkansas (13), Arizona (47), California (8), Colorado (13), Connecticut (4), Florida (2), Georgia (24), Idaho (4), Illinois (95), Indiana (14), Iowa (2), Kansas (17), Kentucky (1), Louisiana (1), Maine (1), Maryland (29), Massachusetts (24), Michigan (7), Minnesota (10), Missouri (12), New Hampshire (4), Nevada (11), New

Jersey (9), New Mexico (98), New York (28), North Carolina (10), Ohio (8), Oklahoma (24), Oregon (10), Pennsylvania (11), Rhode Island (3), South Carolina (1), Tennessee (8), Texas (382), Utah (2), Virginia (29), Vermont (2), Washington (4), West Virginia (1), Wisconsin (11), and the District of Columbia (1). Four ill persons are reported from Canada; three appear to have been infected while traveling in the United States, and one illness remains under investigation.

Among the 711 persons with information available, illnesses began between April 10 and June 25, 2008, including 275 who became ill on June 1 or later. Many steps must occur between a person becoming ill and the determination that the illness was caused by the outbreak strain of *Salmonella*; these steps take an average of 2-3 weeks. Therefore, an illness reported today may have begun 2-3 weeks ago. Patients range in age from <1 to 99 years; 48% are female. The rate of illness is highest among persons 20 to 29 years old; the rate of illness is lowest in children 10 to 19 years old and in persons 80 or more years old. At least 194 persons were hospitalized. One death in a man in Texas in his eighties has been associated with this outbreak. In addition, a man in his sixties who died in Texas from cancer had an infection with the outbreak strain of *Salmonella* Saintpaul at the time of his death; the infection may have contributed to his death

Only 6 persons infected with this strain of *Salmonella* Saintpaul were identified in the country during April through June of 2007. The previous rarity of this strain and the distribution of illnesses in all U.S. regions suggest that the implicated food is distributed throughout much of the country. Because many persons with *Salmonella* illness do not have a stool specimen tested, it is likely that many more illnesses have occurred than those reported. Some of these unreported illnesses may be in states that are not on today□s map.

Health officials have worked continuously since late May to investigate this outbreak. CDC has sent 17 people to the field to work with other public health officials. The investigation is complex and difficult. One difficult aspect is that people often have difficulty remembering exactly what foods they ate, and remembering specific ingredients is even more difficult. Although laboratory testing of fo ds might help, perishable foods that were consumed by ill persons are often not available to test.

Clinical features of *Salmonella* Infection

Most persons infected with *Salmonella* develop diarrhea, fever, and abdominal cramps 12-72 hours after infection. Infection is usually diagnosed by culture of a stool sample. The illness usually lasts 4-7 days. Although most people recover without treatment, severe infections may occur. Infants, elderly persons, and those with impaired immune systems are more likely than others to develop severe illness. When severe infection occurs, *Salmonella* may spread from the intestines to the bloodstream and then to other body sites, and can cause death. In these severe cases, antibiotic treatment may be necessary.

Advice to consumers

At this time, FDA is advising U.S. consumers to limit their tomato consumption to those that are not the likely source of this outbreak. These include cherry tomatoes; grape tomatoes; tomatoes sold with the vine still attached; tomatoes grown at home; and red plum, red Roma, and round red tomatoes from specific sources listed at: http://www.fda.gov/oc/opacom/hottopics/tomatoes.html*. Consumers should be aware that raw tomatoes are often used in the preparation of fresh salsa, guacamole, and pico de gallo, are part of fillings for tortillas, and are used in many other dishes.

C sumers everywhere are advised to:

- Refrigerate within 2 hours or discard cut, peeled, or cooked tomatoes.
- Avoid purchasing bruised or damaged tomatoes and discard any that appear spoiled.
- Thoroughly wash all tomatoes under running water.
- Keep tomatoes that will be consumed raw separate from raw meats, raw seafood, and raw produce items.
- Wash cutting boards, dishes, utensils, and counter tops with hot water and soap when switching between types of food products.

FDA recommends that U.S. retail outlets, restaurants, and food service operators offer only fresh and fresh cut red plum, red Roma, and round red tomatoes and food products made from these tomatoes from specific sources listed at: http://www.fda.gov/oc/opacom/hottopics/tomatoes.html#retailers*. Cherry tomatoes, grape tomatoes, and tomatoes sold with the vine still attached from any source may be offered.

FDA information on this investigation can be found at: http://www.fda.gov/oc/opacom/hottopics/tomatoes.html*

More information about *Salmonella* and this investigation can be found at:

- *Salmonella* in tomatoes FAQs
- Timeline for Reporting of Cases
- New Mexico Department of Health (PDF – 191 KB)
- Arizona Department of Health Services News Release - Tomatoes: Caution Urged*
- Texas Department of State Health Services - News Update, June 13, 2008*
- Kansas Identifies 3 Cases Linked to Multi-State *Salmonella* Outbreak*

- Kentucky Cabinet for Health and Family Services Press Release
- Indiana State Department of Health Media Update on *Salmonella* Outbreak*
- Maryland Department of Health and Mental Hygiene News Release
- Missouri DHHS: State health department issues cautions about tomatoes*
- New Jersey Department of Health and Human Services: NJ Reports Four *Salmonella* Cases Linked to Multi-State Outbreak
- Utah Department of Health: Health News

nformation on the safe handling of produce can be found at: www.cfsan.fda.gov/~dms/prodsafe.html.*

Previous Updates on this Outbreak

- July 7, 2008
- July 4, 2008
- July 3, 2008
- July 2, 2008
- July 1, 2008
- June 30, 2008
- June 27, 2008
- June 26, 2008
- June 25, 2008
- June 24, 2008

- June 23, 2008
- June 20, 2008
- June 18, 2008
- June 16, 2008
- June 12, 2008
- June 9, 2008
- June 7, 2008
- June 5, 2008
- June 2, 2008

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ʾage Located on the Web at http://www.cdc.gov/salmonella/saintpaul/archive/070808.html

別紙様式第2-1
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医薬品
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研究報告の概要

＜背景＞
2005年から2007年の間、チクングニヤウイルス（CHIKV）が、2006年2月に症例数の最大ピークとするレユニオン島での大流行を引き起こした。レユニオン島での供血は、2006年1月に中断された。
＜研究デザインおよび方法＞
レユニオン島でウイルス血症の供血がされる平均リスクの推定を異なる流行期について計算した。計算には、定点観測の動向調査（sentinel surveillance）、ウイルス血症の期間、および無症候感染の頻度から割り出したCHIKV予想発現値を用いた。最後のこれら2つのパラメーターのデータは、最初は仮定に基づき、次いでアウトブレイクの期間に実施された検討をもとに出した。この予想リスクを、血小板ドネーションのスクリーニングのために実施したCHIKV核酸増幅検査の結果と比較した。
＜結果＞
アウトブレイクの期間中、リスクの平均値は、ドネーション100,000当たり132と予想された。このリスクは、2006年2月のアウトブレイクの最大期にピークに達し、ドネーション100,000当たり1,500であった。もし採血が中断されていなかったら、全体で47の供血がウイルス血症であったであろう。この期間、757,000人住民のうちの312,500人が蚊を媒介にして感染していたと予想される。2006年1月から5月まで、予想リスク平均値（0.7%）と血小板供血で観察されたリスク（0.4%）は同じ大きさであった。
＜結論＞
この大きなアウトブレイクの間、ウイルス血症の供血の予想リスクは高かったが、蚊媒介のCHIKV感染のリスクに比べ低かった。この予想リスクは、観察されたリスクの結果と一致したことによって裏付けられた。

使用上の注意記載状況・その他参考事項等

代表として静注用ヘブスブリンーIHの記載を示す。
2. 重要な基本的注意
(1) 本剤の原材料となる血液については、HBs抗原、抗HCV抗体、抗HIV-1抗体、抗HIV-2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高力価の抗HBs抗体を含有する血漿を原料として、Cohnの低温エタノール分画で得た画分からポリエチレングリコール4000処理、DEAEセファデックス処理等により抗HBs人免疫グロブリンを濃縮・精製した製剤であり、ウイルス不活化・除去を目的として、製造工程において60℃、10時間の液状加熱処理及びろ過膜処理（ナノフィルトレーション）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
レユニオン島におけるチクングニヤウイルス（CHIKV）の流行時のCHIKV血症献血リスクに関する報告である。血漿分画製剤からのチクングニヤウイルス伝播の事例は報告されていない。また、万一原料血漿にチクングニヤウイルスが混入したとしても、BVD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。

TRANSFUSION COMPLICATIONS

Estimated risk of Chikungunya viremic blood donation during an epidemic on Reunion Island in the Indian Ocean, 2005 to 2007

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BACKGROUND: Between 2005 and 2007, Chikungunya virus (CHIKV) caused a massive epidemic on Reunion Island with a major peak in the number of cases in February 2006. Blood donation was interrupted on the island in January 2006.

STUDY DESIGN AND METHODS: Estimates of the mean risk of viremic blood donation on Reunion Island were computed for different phases of the epidemic. Calculations used CHIKV incidence estimates derived from sentinel surveillance, duration of viremia, and frequency of asymptomatic infection. Data on these two last parameters were initially based on hypotheses and subsequently obtained from studies carried out during the outbreak. The estimated risk was compared to the results of CHIKV nucleic acid testing (NAT) implemented for platelet (PLT) donations screening.

RESULTS: Over the course of the outbreak, the mean risk was estimated at 132 per 100,000 donations. The risk peaked at 1500 per 100,000 donations at the height of the outbreak in February 2006. In total, 47 blood donations would have been potentially viremic if blood collection had not been interrupted. During this period, an estimated 312,500 of 757,000 inhabitants had been infected by mosquito-borne transmission. From January to May 2006, the estimated mean risk (0.7%) and observed risk on PLT donations (0.4%) were of the same order of magnitude.

CONCLUSION: During this large outbreak, the estimated risk of viremic blood donation was high, but low compared to the risk of mosquito-borne CHIKV transmission. The estimated risk was corroborated by the concordant results with the observed risk.

Chikungunya virus (CHIKV) is an alphavirus that belongs to the Togoviridae family, transmitted by *Aedes* mosquitoes. It was first identified in 1952 during an outbreak in Tanzania.[1,2] Afterward, it caused many outbreaks in Africa[3-7] and in Asia.[3,8-11] In Africa, a sylvatic transmission cycle between wild primates and mosquitoes is thought to maintain the virus, whereas in Asia, it is transmitted from human to human through an urban transmission cycle.[3] CHIKV infection is mainly characterized by sudden onset of fever, arthralgia, myalgia, headache, and edemas.[1,3,8,12,13] Other symptoms like rash, epistaxis, gingivorrhagia, nausea, vomiting, flushed face, or photophobia have also been described. The most typical clinical sign is polyarthralgia that is generally very painful, as suggested by its name Chikungunya meaning in the language of the Tanzanian Makonde plateau "that which bends up" in reference to the stooping posture adopted by patients because of the severity of the joint pains. The symptoms usually resolve within a few days, but in some severe cases, arthralgia may persist for months or years.[3,13] Serosurveys implemented during prior outbreaks have demonstrated that Chikungunya infection can also be asymptomatic.[9]

In early 2005, CHIKV emerged for the first time in the southwest Indian Ocean region (Comoros, Reunion,

ABBREVIATIONS: CHIKV = Chikungunya virus; WNV = West Nile virus.

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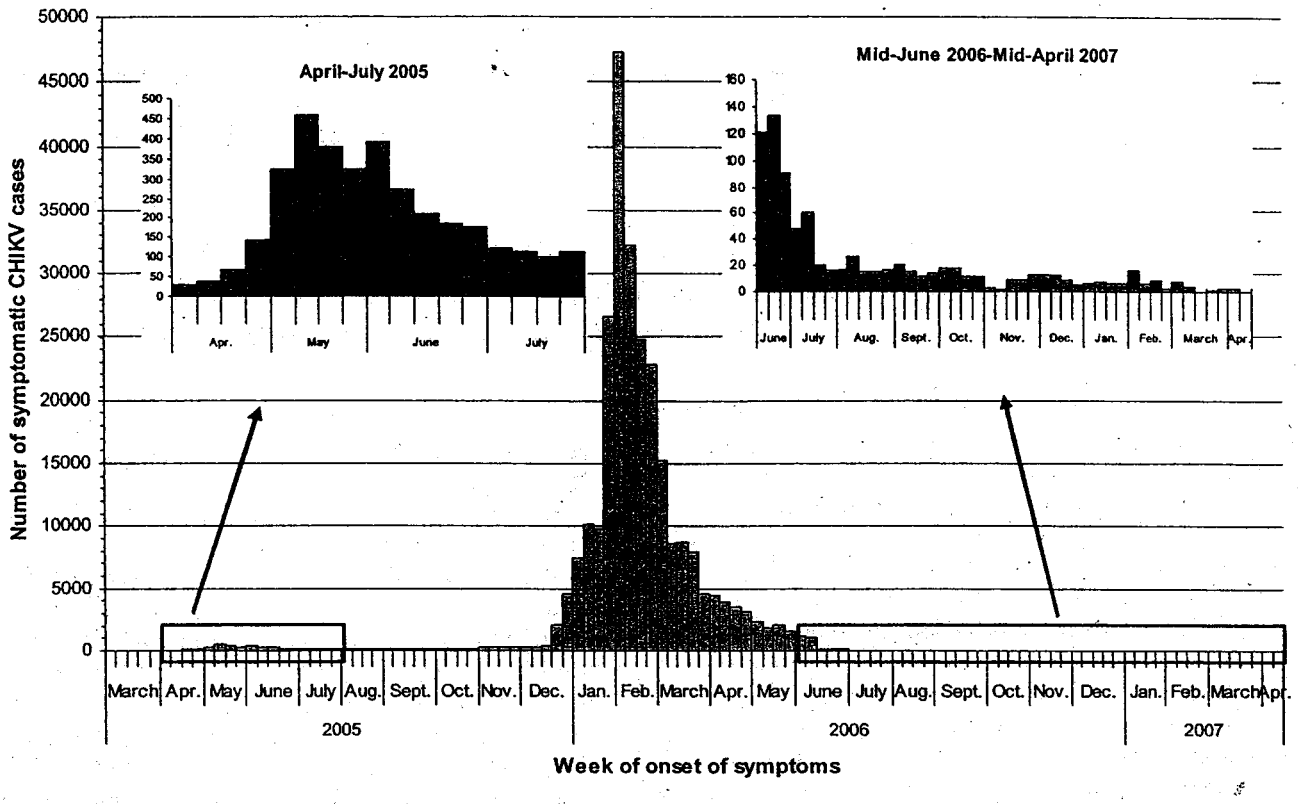

Fig. 1. Distribution of symptomatic cases of CHIKV infection per week of onset of symptoms, Reunion Island, March 28, 2005, through April 15, 2007.

Mayotte, Seychelles, Mauritius, and Madagascar Islands). On Reunion Island, the first cases were identified at the end of April 2005. After a first epidemic peak in May through June 2005 with a maximum of 450 cases during the second week of May, the number of cases decreased during the southern hemisphere winter season. At mid-December, an exponential increase in cases occurred, with almost 10,000 estimated cases at mid-January 2006 (Fig. 1). Because of concerns about the possible transmission of CHIKV by blood transfusion, the French Blood Services (EFS) interrupted blood donations on the island from January 23, 2006, except donations for platelets (PLTs) for which systematic screening for CHIKV genome by nucleic acid amplification testing (NAT) was set up.

At that moment, we estimated the risk of CHIKV viremic blood donation. Afterward, we updated these estimates since more accurate data were available on the incidence of infection and on the frequency of asymptomatic infections. We compared the estimated risk of viremic blood donation to the observed proportion of viremic PLT donations determined by CHIKV NAT screening.

MATERIALS AND METHODS

The estimates were performed by the French Institute of Public Health Surveillance (InVS) in the setting of a work-group including the French Agency for the Safety of Health Products (Afssaps), the French Blood Services (EFS), and the National Institute for Blood Transfusion (INTS). In early 2005, this group initiated a project with the aim of obtaining a priori quantitative risk estimates of contamination of blood donations by infectious agents for various scenarios in terms of incidence and time-space distribution.[14]

General approach

The first estimates performed in January 2006 ("preliminary estimates") concerned the two following periods: Period A, from the detection of the first cases in April 2005 to mid-December 2005 when a large increase of cases occurred (March 28-December 18, 2005; 266 days); and Period B, from mid-December until the interruption of blood collection (December 19, 2005-January 22, 2006; 35 days; Fig. 2).

These estimates were later refined with consolidated incidence data, corrected for delayed care-seeking and delayed reporting and more precise estimates of the proportion of asymptomatic infections obtained through a seroepidemiologic survey carried out at the final phase of the outbreak ("retrospective estimates"). We also estimated the risk of viremic blood donation for five different

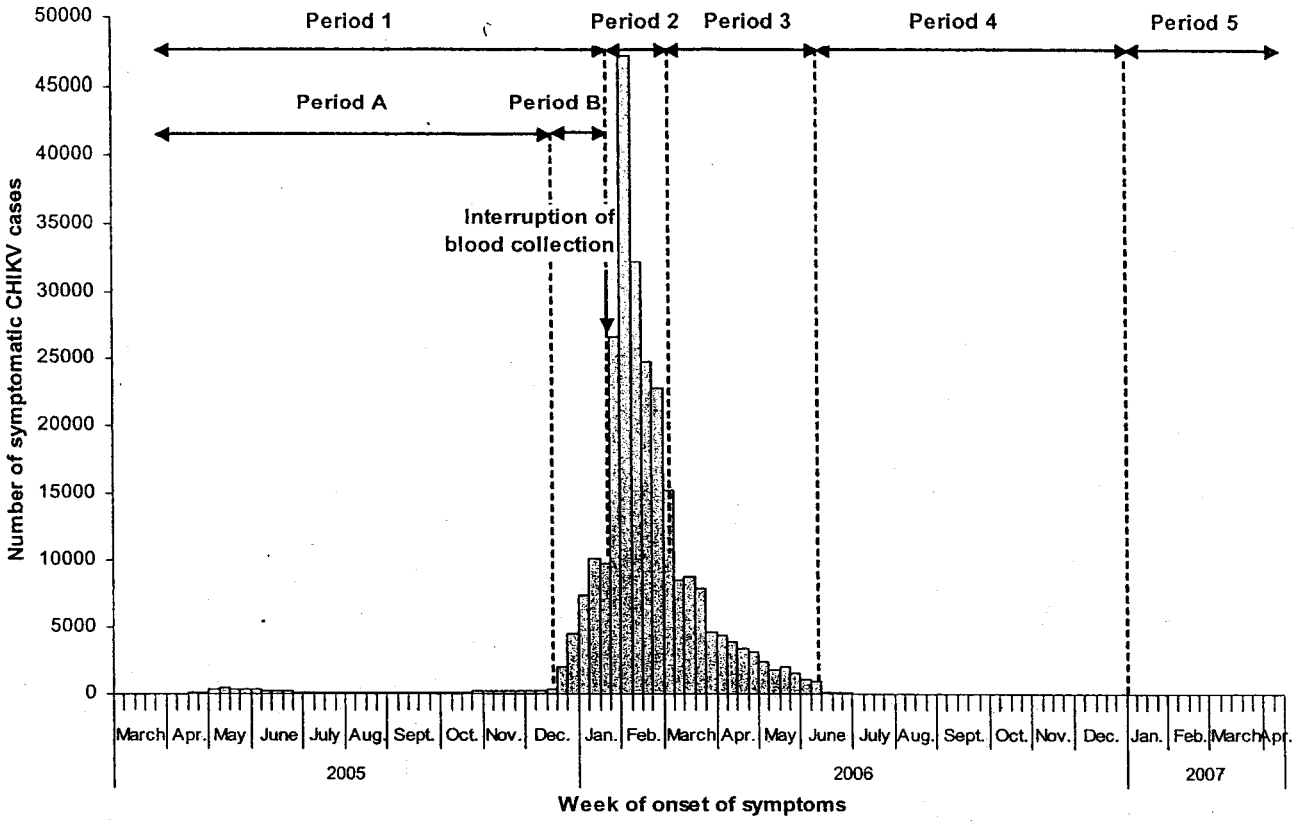

Fig. 2. Periods for risk estimates and distribution of symptomatic cases of CHIKV infection per week of onset of symptoms, Reunion Island, March 28, 2005, through April 15, 2007.

periods of the outbreak with these updated data (Fig. 2).

By use of the quarterly numbers of blood donations collected on Reunion Island in 2005 (unpublished data from EFS), we could then estimate the number of blood donations that would have been collected in 2006 if blood donations had not been interrupted.

To assess the validity of our risk estimates, we compared the estimated risk of viremic blood donation ("estimated risk") to the observed proportion of viremic PLT donations collected and screened for CHIKV genome ("observed risk") over the same period.

Statistical approach

An approximating formula developed by Biggerstaff and Petersen[15] in 2002 for West Nile virus (WNV) was used to estimate the mean risk of viremic blood donation by CHIKV. This formula combines the proportion of asymptomatic (*Pa*) and symptomatic (*Ps*) infections with the duration of viremia among asymptomatic infected individuals (*Va*) and the duration between onset of viremia and onset of symptoms in symptomatic patients (*Vs*). This provides the mean time an infected individual is viremic and asymptomatic. Dividing this mean duration of viremia by the length of the outbreak period (*L*) then provides an estimate of the probability that an individual donates blood during viremia, assuming that a person with symptoms would self-defer or be excluded from donation by the predonation medical examination. Combined with the incidence (*I*) of the infection (including both symptomatic and asymptomatic infection), it gives an estimate of the mean risk of viremic blood donation:

$$\text{Mean risk} = \frac{(Pa \times Va) + (Ps \times Vs)}{L} \times I.$$

As suggested by Biggerstaff and Petersen,[15] risk confidence bounds were obtained by multiplying the confidence bounds of *I* by $[(Pa \times Va) + (Ps \times Vs)]/L$. Confidence intervals (CIs) of *I* were calculated with Fleiss quadratic method.[16]

Data on duration of viremia

In January 2006, few data were available on the duration of CHIKV viremia. In 1964, Sarkar and coworkers[17] described, from virologic studies of hemorrhagic fever in Calcutta, that CHIKV was most frequently isolated from blood within 48 hours after the onset of symptoms, but that it had been isolated as late as 6 days after the onset of illness.

The duration of viremia has been more extensively documented for dengue viruses: 1 or 2 days before the onset of symptoms and between 4 and 6 days and as late as 12 days after the first symptoms.[18-20] We thus used the following parameters for CHIKV: 1.5 days for the mean duration between onset of viremia and onset of symptoms among symptomatic patients (*Vs*) and 1.5 + 6 = 7.5 days for the mean duration of viremia among asymptomatic infected individuals (*Va*) assuming that the whole duration of viremia is similar in symptomatic and asymptomatic infections.

The same estimates of duration of viremia were used for the retrospective estimates since consistent observations were reported during the outbreak on Reunion Island. Thus, during this epidemic, CHIKV has been isolated from blood mostly within 5 days and as late as 12 days after the onset of symptoms. In some cases, CHIKV viremia might have persisted over 12 days since viral loads at 12 days were high.[21]

Data on the proportion of asymptomatic infections

For the preliminary estimates in January 2006, in the absence of data on the proportion of asymptomatic CHIKV infection, two hypotheses were formulated based on the proportion of asymptomatic infections reported during outbreaks of dengue:[22,23] a minimal proportion of asymptomatic infection of 30 percent and a maximal proportion of 70 percent.

Between August and October 2006, a seroprevalence study was conducted among the general population of Reunion Island.[24] This survey showed that 38 percent of the inhabitants of Reunion Island had been infected by CHIKV. The preliminary results indicated that 6 percent of the study population had a positive CHIKV serology without having reported CHIKV symptoms. This suggests that approximately 15 percent of infected individuals during this outbreak may have had an asymptomatic infection. Therefore, this proportion of 15 percent was used for *Pa* for the retrospective estimates.

Incidence of CHIKV infection

We used the incidence data in the general population for the risk estimations assuming that potential blood donors had the same risk of CHIKV infection as the general population. The population of interest was the inhabitants of Reunion Island estimated at 756,745 by a population census conducted in 2004 by the National Institute for Statistics and Economics Studies (INSEE). CHIKV incidence data, by week of onset of symptoms, were obtained from the Reunion-Mayotte Interregional Epidemiology Unit, which had started surveillance for CHIKV infection as soon as the first cases were reported in April 2005. A suspect case of CHIKV infection was defined as a patient with an abrupt onset of fever over 38.5°C associated with incapacitating arthralgia in the absence of any other potential cause of infection. From April to December 2005, surveillance relied on vector control teams, which conducted active and retrospective case-finding around the cases reported by a sentinel physician network, medical laboratories, private practitioners, and patients themselves. The number of cases took into account the symptomatic patients responding to the case definition whether or not they had consulted a general practitioner. During this period, approximately 67 suspect CHIKV cases were identified by active case-finding for every suspect case identified by the sentinel network physicians. From mid-December onward, the number of cases exceeded the capacity of the active surveillance system, and surveillance was then entirely based on the sentinel network. To estimate the total number of cases from the sentinel network data, the multiplier of 67, derived during the phase of active case finding, was used.[25]

For the estimations of the risk of viremic donations, we calculated the estimated incidence of symptomatic and asymptomatic CHIKV infection by multiplying the estimated incidence of suspect cases by 100/(proportion of symptomatic infections).

RESULTS

Preliminary estimates

When the preliminary estimates were performed at the end of January 2006, the number of CHIKV suspect cases was 6500 for Period A and 25,000 for Period B. For Period A, the estimated mean risk of viremic blood donation was 15.2 per 100,000 donations, under the minimal hypothesis of 30 percent asymptomatic infections, and 61.3 per 100,000 donations, under the maximal hypothesis of 70 percent asymptomatic infections (Table 1). For Period B, the mean risk reached 445 per 100,000 donations, under the minimal hypothesis and 1,793 per 100,000 donations, under the maximal hypothesis.

Retrospective estimates

The retrospective estimates used the results of the seroprevalence survey that estimated the proportion of asymptomatic CHIKV infections during this outbreak at 15 percent. The updated estimate of the number of symptomatic cases was 6,864 for Period A and 34,002 for Period B (Table 2). Risk of viremic blood donation was then estimated at 9.6 and 362.5 per 100,000 donations for Periods A and B, respectively. The risk estimates for the five periods of the outbreak are shown in Table 3. Between the identification of the first CHIKV cases and the interruption of blood donations (Period 1), 7 of 14,450 blood donations collected could have been viremic. During

TABLE 1. Preliminary risk estimates of viremic blood donation, Reunion Island, March 28, 2005, through January 22, 2006

	Period A, Mar 28-Dec 18, 2005		Period B, Dec 19, 2005-Jan 22, 2006	
	Minimal hypothesis	Maximal hypothesis	Minimal hypothesis	Maximal hypothesis
Estimated number of symptomatic cases	6,500	6,500	25,000	25,000
Proportion of asymptomatic infections (%)	30	70	30	70
Estimated number of infected cases	9,286	21,667	35,714	83,333
Period length (days)	266	266	35	35
Estimated incidence of CHIKV infection per 100,000	1,227	2,863	4,720	11,012
Estimated risk of viremic blood donation				
Per 100,000 blood donations (95% CI)	15.2 (14.9-15.5)	61.3 (60.6-62.2)	445.0 (440.5-449.5)	1,793.4 (1,781.9-1,804.9)
Per estimated number of blood donations (95% CI)	2.0/12,800 (1.9-2.0)	7.9/12,800 (7.8-8.0)	7.1/1,600 (7.0-7.2)	28.7/1,600 (28.5-28.9)

TABLE 2. Retrospective risk estimates of viremic blood donation, Reunion Island, March 28, 2005, through January 22, 2006

	Period A, Mar 28-Dec 18, 2005	Period B, Dec 19, 2005-Jan 22, 2006
Estimated number of symptomatic cases	6,864	34,002
Proportion of asymptomatic infections (%)	15	15
Estimated number of infected cases	8,075	40,002
Period length (days)	266	35
Estimated incidence of CHIKV infection per 100,000	1,067	5,286
Estimated risk of viremic blood donation		
Per 100,000 blood donations (95% CI)	9.6 (9.4-9.8)	362.5 (359.0-366.0)
Per estimated number of blood donations (95% CI)	1.2/12,800 (1.2-1.3)	5.8/1,600 (5.7-5.9)

Period 2, at the height of the epidemic, the estimated risk of viremic blood donation was 1,500 per 100,000, that is, 29 potentially viremic donations if blood collection had continued. The estimated risk then decreased due to diminishing CHIKV transmission: 210 per 100,000 between March and June 2006 (Period 3), 1.4 per 100,000 for the second semester of 2006 (Period 4), and 0.27 per 100,000 for the first months of 2007 (Period 5), that is, 1 potentially viremic blood donation every 21 years on the basis of 17,500 blood donations collected each year. Finally, over the course of the outbreak, a total of 47 of 35,750 blood donations might have been viremic if blood collection had continued. Simultaneously, an estimated 312,500 of 757,000 inhabitants have been infected by mosquito-borne transmission.

Comparison between estimated risk and observed risk

Between January 23 and May 7, 2006, 2 of the 500 PLT donations screened for CHIKV RNA were positive (0.4%). One donor developed CHIKV symptoms on the day after the blood donation, the other remained asymptomatic. The risk of viremic blood donation over this period was estimated at 720 per 100,000 blood donations, that is, 0.72 percent.

Although an estimated 7 viremic donors had donated blood before the collection was interrupted, no case of transfusion-transmitted CHIKV infection has been identified during this period.

DISCUSSION

During this first and massive epidemic of CHIKV infection on Reunion Island, we computed estimates of the risk of CHIKV viremic blood donation, in real time during the ascending phase of the major epidemic peak, and afterward, we refined these estimates with newly available data. Although we underestimated the incidence of CHIKV infection in our preliminary calculations, we overestimated the proportion of asymptomatic infections. Consequently, the preliminary estimates were 1.2- to 6.4-fold greater than the retrospective calculations. The preliminary estimates, however, provided a right order of magnitude of the risk in real time in an emergency context. The retrospective calculations indicate a mean risk over the course of the outbreak, between April 2005 and April 2007, of 132 per 100,000 donations. The mean risk peaked at approximately 1,500 per 100,000 donations at the height of the outbreak in February 2006. In total, potentially 47 of 35,750 blood donations might have been viremic between April 2005 and April 2007 if blood collection had not been interrupted. We also estimated that 7 blood donations were viremic before the interruption of blood donations on the island. Therefore, this measure enabled the avoidance of 40 potentially viremic donations. By way of comparison, during the outbreak, the total number of individuals infected through mosquito-borne CHIKV transmission is estimated at 312,538 individuals.

This approach has several limitations. The estimates provided relate to a mean risk, which supposes that the risk is constant over the studied period and for the

TABLE 3. Retrospective risk estimates of viremic blood donation, Reunion Island, March 28, 2005, through April 15, 2007

	Period 1, Mar 28, 2005-Jan 22, 2006	Period 2, Jan 23, 2006-Mar 5, 2006	Period 3, Mar 6, 2006-Jun 11, 2006	Period 4, Jun 12, 2006-Dec 31, 2006	Period 5, Jan 1, 2007-Apr 15, 2007	Periods 1-5, Mar 28, 2005-Apr 15, 2007
Estimated number of symptomatic cases	40,866	169,008	54,936	772	75	265,657
Period length (days)	301	42	98	203	105	749
Proportion of asymptomatic infections (%)	15	15	15	15	15	15
Estimated number of infected cases	48,078	198,833	64,631	908	88	312,538
Estimated incidence of CHIKV infection per 100,000	6,353	26,275	8,541	120	12	41,300
Estimated risk of viremic blood donation						
Per 100,000 blood donations (95% CI)	50.7 (50.2-51.1)	1,501.4 (1,495.8-1,507.1)	209.2 (207.6-210.7)	1.4 (1.3-1.5)	0.27 (0.2-0.3)	132.3 (132.0-132.7)
Per estimated number of blood donations (95% CI)	7.3/14,450 (7.3-7.4)	29.1/1,940 (29.0-29.2)	9.9/4,710 (9.8-9.9)	0.14/9,760 (0.13-0.15)	0.01/4,890 (0.01-0.02)	47.3/35,750 (47.2-47.4)

geographic area. Although estimates were performed for several periods selected according to the level of incidence, the number of cases and consequently the risk might have been highly variable during the studied period. In addition, the risk of infection varied by geographic area as later demonstrated by the seroprevalence survey that showed that 29.6 percent of the inhabitants of the North have been infected whereas in the East, this proportion reached 48 percent.[24] Consequently, the mean risk underestimates the maximal risk, corresponding to the peak of the outbreak and to the area where CHIKV transmission was maximal. This maximum risk, however, is highly time and space limited.

To obtain a more dynamic sight of the risk over the course of the epidemic and estimates of the maximal risk, it would have been necessary to develop an approach similar to the one proposed by Biggerstaff and Petersen[15,26] for the WNV epidemic in 2002 in the United States. The latter is a statistical approach based on imputation and resampling techniques providing daily estimates of the risk of blood contamination in an epidemic setting. Conducting such an analysis in the context of this large and long-standing outbreak would have been computationally cumbersome. In our opinion, such a refinement was not essential in regard to the main objectives of the study, that is, providing a right order of magnitude of the risk as an aid for risk management. We considered that providing an approximation of the mean risk over five periods was a suitable alternative. To compute these mean risks, we therefore used the approximating formula proposed by Biggerstaff and Petersen.[15] In 2003, Biggerstaff and Petersen demonstrated for the WNV epidemic in 2002 in the United States that the approximating formula provides a reasonable approximation to the mean risk of transfusion.[15] The same work of comparison of the mean risks estimated by this method and by statistical resampling was carried out, in the setting of our workgroup, for an outbreak of acute hepatitis A in France that occurred in 1996 through 1997.[14,27] It also concluded to a good concordance of the results of both methods. Note that the CIs presented with our mean risk estimates do not take into account the uncertainty on the duration of viremia, the proportion of asymptomatic infections, nor the coefficient of 67, used to estimate incidence of symptomatic infections from the sentinel network data. Even though this limitation led to artificially narrow CIs, point estimates of mean risk should not be affected.

Our incidence data were derived from a sentinel surveillance system. Because a clinical case definition was used, it is possible that other febrile illnesses, not due to CHIKV, were included in the case count. The positive predictive value of a clinical case definition, however, greatly improves if incidence is high. Therefore, the inclusion of noncases in the case count, leading to

overestimation of the incidence and hence the risk of viremic donation, is more likely to occur outside an outbreak setting. The validity of the extrapolated data derived from a sentinel surveillance system estimating the total number of cases in the community should also be questioned. The serosurvey estimated that 38 percent of the inhabitants had been infected and that 32 percent had suffered from symptomatic infections. These data are consistent with the 35 percent of the inhabitants having suffered from symptomatic illness, estimated by the sentinel surveillance system and corroborate our incidence estimates.

We assumed that potential blood donors had the same risk of CHIKV infection as the general population. This assumption was supported by the findings of the serosurvey that showed similar antibody prevalences among adults of both sexes.[24] In addition, when we applied age-specific CHIKV antibody prevalence rates of the serosurvey to the donor population of Reunion Island, the overall seroprevalence among donors was estimated at 37.2 percent, similar to the overall antibody prevalence in the general population (38%).

One major limitation of the validity of our estimates relates to lack of a precise knowledge on the distribution of the duration of asymptomatic viremia in individuals with apparent and inapparent infection. To refine the estimates, further studies are necessary to document the kinetics of CHIKV viremia. This approach also hypothesizes that symptomatic individuals would self-defer or be excluded by the predonation examination. In real life, this may not always be the case. In the United States, among the first 14 identified donors associated with transfusion-related WNV transmission to recipients, 3 were shown to have been symptomatic at the moment of the donation.[15] Nevertheless, for CHIKV infection which is characterized by sudden onset of symptoms, this assumption is more plausible than for WNV which frequently causes paucisymptomatic infection.

Lack of data on the frequency of asymptomatic infection was the most important limiting factor for the preliminary estimates. This variable has a preponderant role in the risk estimate since it contributes both in the computation of the weighted mean of the duration of asymptomatic viremia and in the estimate of the incidence of infection. Valid data were available, however, for the retrospective calculations from the seroprevalence survey. This survey provided an estimate of the proportion of asymptomatic infections obtained directly among the studied population and for the epidemic CHIKV strain circulating.

In spite of the above limitations, the retrospective estimates are likely to give a good approximation of the real risk, as suggested by the observed risk of viremic PLT donations. From January to May 2006, this observed risk was 400 per 100,000 donations, of the same order of magnitude as the risk of 720 per 100,000 donations estimated over the same period.

Up to date, CHIKV infections from transfusion of blood or blood components have not been reported in the literature. On Reunion Island, no case of transfusion-transmitted CHIKV infection has been identified in spite of the estimated seven viremic donations collected before donations were interrupted. Despite the lack of data about transfusion-transmitted CHIKV infection, the high viral load during the acute phase of the infection,[21,28] the fact that several cases of CHIKV transmission have occurred among laboratory personnel handling infected blood,[29] and the fact that CHIKV has been transmitted to a health care worker drawing blood from an infected patient[28] provide evidence that transfusion-related transmission of CHIKV is highly plausible. It is possible that transfusion-related infections have not been recognized or have not been distinguished from infection from mosquito vectors. Also, the true transmission rate from viremic donors to recipients is not known. Several issues may influence the possibility of transmission of CHIKV through transfusion, such as the stability of the virus during storage of blood and the efficiency of virus elimination of blood processing methods, as viral inactivation. Also, the presence of IgM or IgG antibodies in donor blood may neutralize infectivity, as demonstrated for other viruses such as parvovirus[30] and suggested for WNV.[31] In addition, the assessment of the risk of CHIKV transmission from a viremic donor to a recipient would need to take into account the recipient's immune status.

In conclusion, despite the absence of documented cases, blood transfusion–related CHIKV transmission is plausible and the risk of viremic donation can be substantial in an outbreak setting. During this large outbreak, the estimated risk of viremic blood donation was high, but low compared to the risk of mosquito-borne CHIKV transmission. Despite its limitations, this work provided a right order of magnitude of the risk of viremic blood donation in real time during the ascending phase of the epidemic peak. At this moment, the decision of interrupting blood collection relied on the precautionary principle. The low risk estimated for early 2007 was, however, useful to contribute to the decision making process to start again the collection of blood donations on the island from June 14, 2007. This illustrates how this approach may contribute to guiding prevention measures.

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研究報告の概要

○1998年～2005年の香港におけるデング熱症例の検討
目的:デング患者の疫学的、臨床的知見、臨床検査知見、並びに転帰の検討。
患者:1998年～2005年に香港の公立病院に入院したデング患者(臨床検査による確定例)全員の医療記録を後方視的に検討した。
結果:合計126名の患者を特定した[デング熱123名(98%)、デング出血熱3名(2%)]。輸血によりデング熱が伝播した患者1名が明らかとなった。合計116名(92%)は「輸入感染」で、10名(8%)は「地域内感染」であった。RT-PCRで確定したデング症例56名のうち、もっとも多かったのはデングウイルス1型(48%)であり、ついで2型(23%)3型(16%)、4型(13%)であった。地域内感染は1、2型のみであった。患者の年齢の中央値は38歳で、入院期間の平均は6日間であった。死亡例はなく、ほぼ全員(98%)が発熱を呈した。入院時のその他の症状は次の通り:筋肉痛(83%)、頭痛(65%)、倦怠感(59%)、皮疹(60%)。3分の1以上の患者が胃腸および上気道の合併症を発現した。もっとも多く認められた身体的所見は斑丘疹状皮疹であった。血小板減少、好中球減少、リンパ球減少は、それぞれ86%、78%、69%の患者に発現した。人口統計学的・臨床的知見、臨床検査知見、ならびに転帰は、4つのデング血清型間で差はなかったが、リンパ球数は、他の型と比べて3型がもっとも低かった(P=0.004)。
結論:発熱、皮疹を呈し、合致する血液学的知見を持ち、流行地への渡航歴のある患者に遭遇した場合には鑑別診断にデング熱を含めるべきである。

使用上の注意記載状況・その他参考事項等

合成血-LR「日赤」
照射合成血-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

報告企業の意見	今後の対応
1998年～2005年に香港の公立病院に入院したデング患者は合計126名で、うち10名(8%)は「地域内感染」であり1名は輸血による感染だった。ウイルス型は1型が最も多く、地域内感染は1、2型のみであったとの報告である。デングウイルスは東南アジアに定着しており、中国や台湾など日本に近い地域での流行状況を注視していく必要がある。	日本赤十字社では、輸血感染症対策として問診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。問診でデング熱の既往があった場合には、治癒後1ヶ月間献血不適としている。また、厚生労働科学研究「献血血の安全性確保と安定供給のための新興感染症等に対する検査スクリーニング法等の開発と献血制限に関する研究」班に協力する予定である。今後も引き続き情報の収集に努める。

ORIGINAL ARTICLE

Review of dengue fever cases in Hong Kong during 1998 to 2005

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CME

Objective To describe the epidemiology, clinical and laboratory findings, and outcomes of patients presenting locally with dengue.

Design Retrospective review of case records.

Setting Public hospitals, Hong Kong.

Patients Medical records of all laboratory-confirmed dengue patients admitted to public hospitals during 1998 to 2005 were reviewed retrospectively.

Results A total of 126 cases were identified, 123 (98%) being dengue fever and three (2%) dengue haemorrhagic fever. One patient who had blood transfusion–acquired dengue fever was highlighted. A total of 116 (92%) cases were 'imported', while 10 (8%) were local. Among the 56 dengue cases confirmed by reverse transcription–polymerase chain reaction, dengue virus type 1 was the most common accounting for 48% of them, followed by type 2, type 3, and type 4 responsible for 23%, 16%, and 13%, respectively. Only type 1 and type 2 were present in locally acquired infections. The median age of the patients was 38 years and the mean duration of hospitalisation was 6 days. There was no mortality, and nearly all patients (98%) presented with fever. Other symptoms at presentation included: myalgia (83%), headache (65%), fatigue (59%), and skin rash (60%). More than one third of patients had gastro-intestinal and upper respiratory complaints. Maculopapular skin rash was the most common physical finding. Thrombocytopenia, neutropenia, and lymphopenia were present in 86%, 78%, and 69% of the patients, respectively. In only 29% of the patients was dengue fever included in the initial differential diagnosis. The demographic, clinical, and laboratory findings as well as outcomes did not differ significantly among the four dengue serotypes, but the lowest lymphocyte counts of type 3 was lower than the other serotypes (P=0.004).

Conclusion When physicians encounter patients with a relevant travel history, presenting with fever and skin rash, and having compatible haematological findings, dengue fever should be included in the differential diagnosis.

Key words
Dengue; Dengue hemorrhagic fever; Serotyping

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Introduction

Dengue is the most common and widespread arthropod-borne viral infection in the world today. It is recognised in over 100 countries throughout the tropics and subtropical areas and threatens the health of approximately 40% of the world's population, of nearly 2.5 billion people.[1] The highest burden of disease occurs in South-East Asia and the Western Pacific, where it is one of the 10 leading causes of hospitalisation and childhood mortality.[2]

In Hong Kong, dengue fever was made notifiable since March 1994 and all infections reported to the Department of Health (DH) are investigated to establish their source. The number of cases reported is showing an increasing trend in recent years; the vast majority being imported from other countries. Hong Kong experienced its first local dengue case in September 2002.[3] Thereafter, several others were encountered in Ma Wan and local cases were subsequently identified sporadically in 2002 and 2003.

The epidemiology, clinical manifestations, and laboratory findings of dengue fever infections and its complications have been extensively described in the medical literature,[4,5] but comprehensive review is lacking for our local patients.

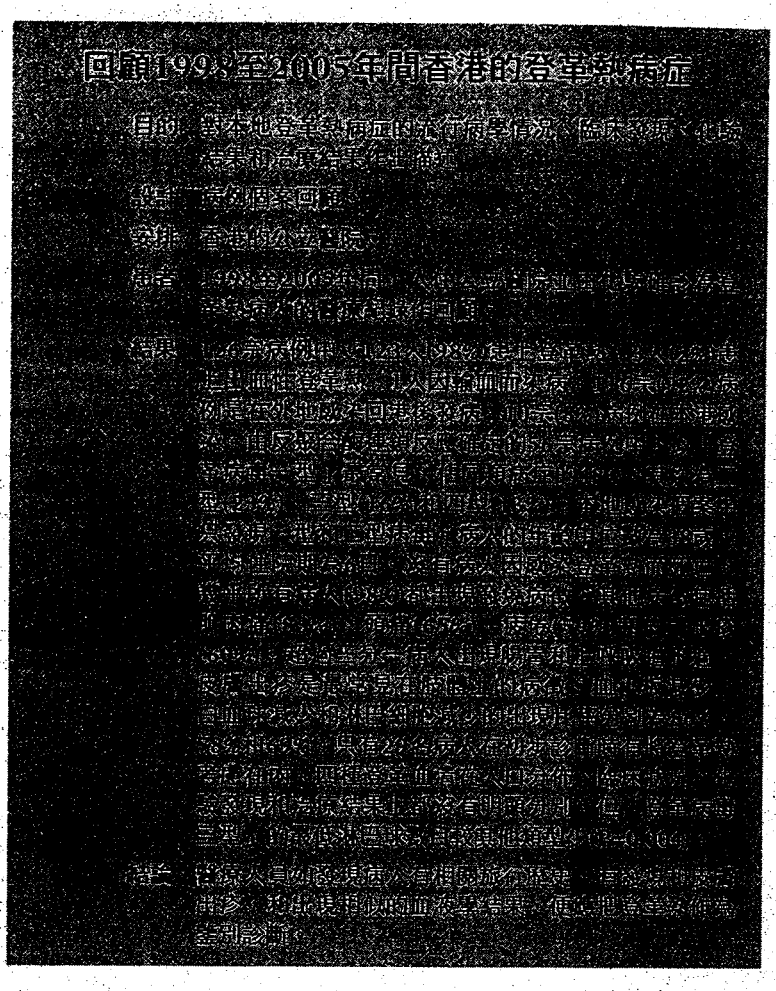
The objective of this review was to describe the epidemiology and explore the clinical characteristics and laboratory findings of dengue fever and dengue haemorrhagic fever (DHF) cases admitted to Hong Kong public hospitals during the period 1998 to 2005. We also compared the clinical and laboratory features of the four dengue serotypes identified by the polymerase chain reaction (PCR) technique.

Methods

We included patients admitted to public hospitals during 1998 to 2005 by using selective criteria "any diagnosis ICD9CM code" starting with "061 dengue" through the Clinical Data Analysis and Reporting System. A patient list was retrieved and matched with the laboratory-confirmed dengue cases notified to the DH. A case was defined as confirmed by detection of viral genomic sequences in autopsy tissue, serum or cerebrospinal fluid samples by PCR; a four-fold or more rise in immunoglobulin G (IgG) or IgM antibody titres to one or more dengue virus antigens in paired serum samples; or a positive IgM antibody titre in late acute or convalescent phase serum specimens (obtained between September 2003 and July 2004). The epidemiological data and virological results were provided by the Surveillance and Epidemiology Branch, Centre for Health Protection, DH. The clinical presentations, laboratory findings, and outcomes of all the confirmed cases were retrospectively reviewed through medical records.

The dengue cases were categorised into dengue fever, DHF, and dengue shock syndrome. In this paper, the definition of DHF was based on the World Health Organization's criteria and defined as: fever lasting 2 to 7 days, haemorrhagic tendencies (a positive tourniquet test; petechiae, ecchymoses or purpura; bleeding from the mucosa, gastro-intestinal tract, injection sites or other locations; haematemesis or melaena), thrombocytopenia (with platelet counts $\leq 100 \times 10^9$ /L) and evidence of plasma leakage due to increased vascular permeability (a rise in haematocrit ≥20% above average for age, sex in the population, a drop in the haematocrit following volume-replacement treatment of ≥20% from baseline, and features consistent with plasma leakage such as pleural effusion, ascites, and hypoproteinaemia). Dengue shock syndrome was defined as DHF together with direct evidence of circulatory failure or indirect evidence manifested as a rapid and weak pulse, narrow pulse pressure (20 mm Hg or hypotension for age) or cold, clammy skin and altered mental status.

Statistical analysis was carried out to compare the epidemiological, clinical, and laboratory findings among the four dengue serotypes. The categorical variables were compared by the Chi squared and Fisher's exact tests. Normally distributed data were compared by analysis of variance and data with skewed distributions by the Kruskal-Wallis test.



Results

Disease trend

In all, 126 patients with laboratory-confirmed dengue fever were admitted to public hospitals from 1998 to 2005. Only three (2%) patients suffered from DHF, while the remaining 123 (98%) had dengue fever; no dengue shock syndrome was reported. The number of patients encountered showed an upward trend from 1998 (2 cases) to 2003 (35 cases), and subsequently remained more or less constant in 2004 (20 cases) and 2005 (24 cases). A total of 116 (92%) were imported, while in 10 (8%) the infection was locally acquired (Fig 1).

No locally acquired disease was reported until in 2002, when nine patients were identified. Among them, six cases were confirmed to be epidemiologically related to the Ma Wan outbreak. Another patient acquired the infection through blood transfusion from one of the Ma Wan cases. The remaining two locally acquired cases in 2002 and one

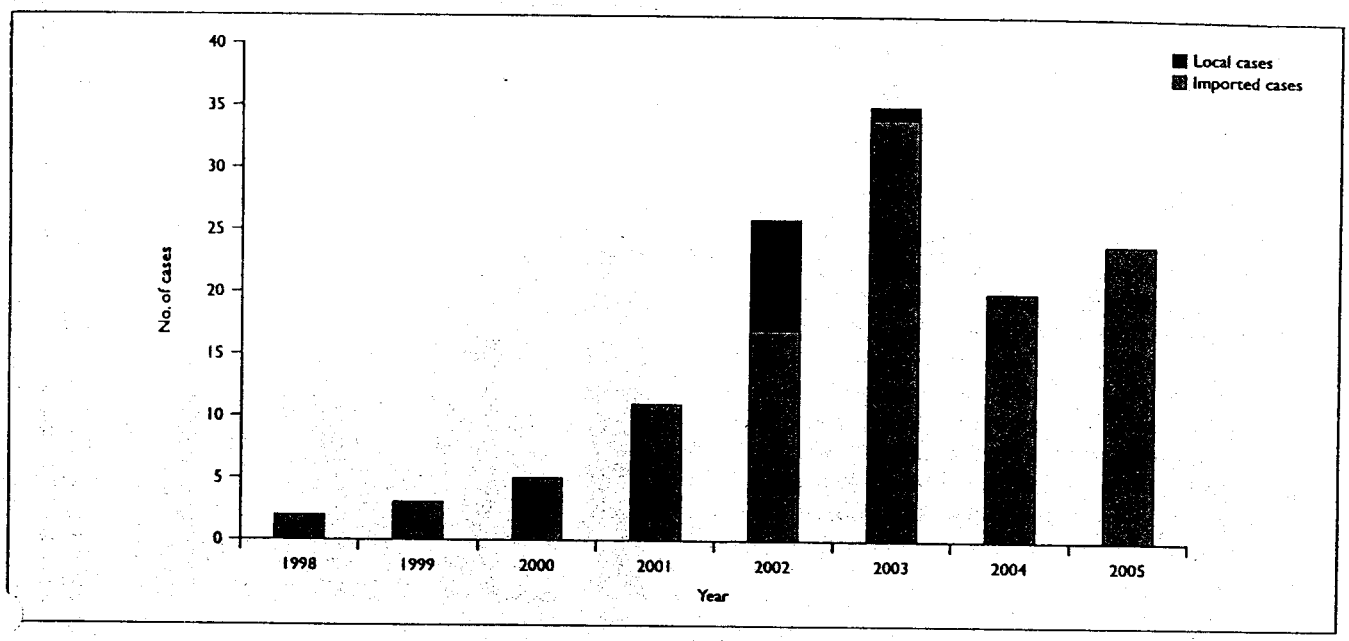


FIG 1. Numbers of dengue fever cases admitted to public hospitals in Hong Kong, 1998-2005

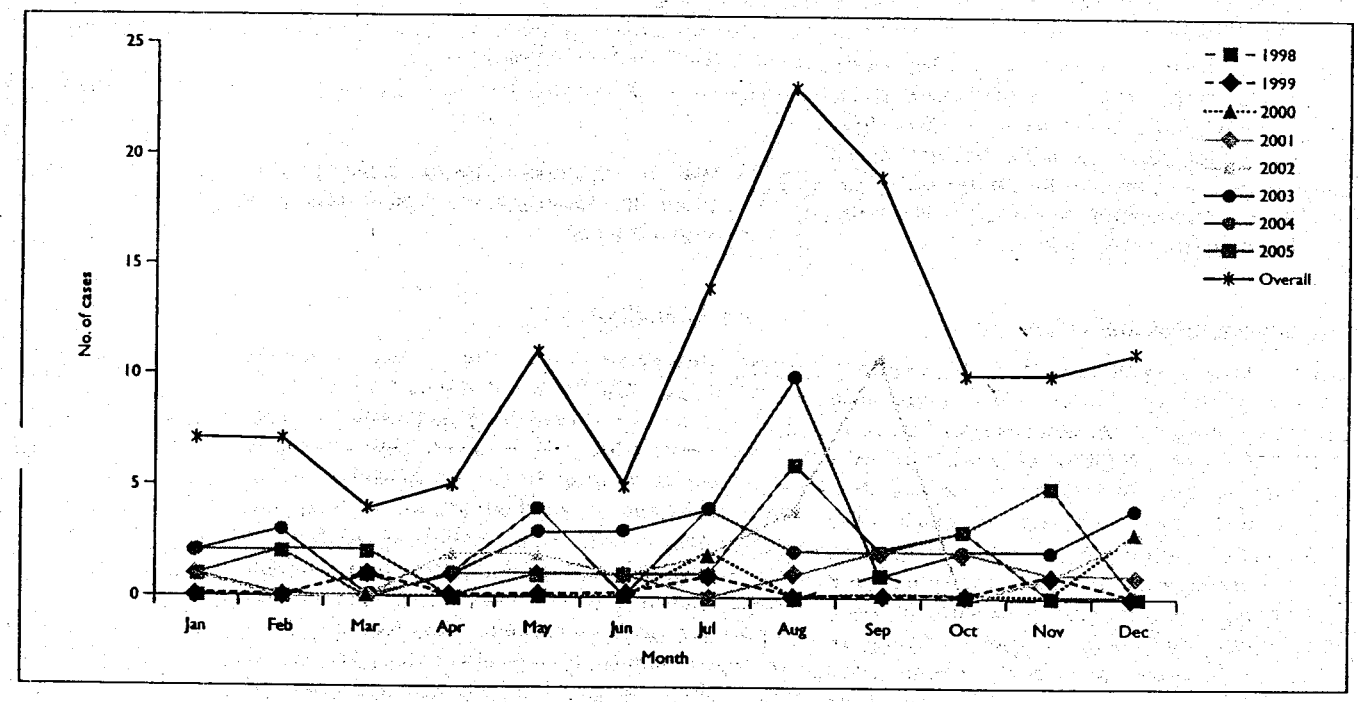


FIG 2. Seasonal variation of dengue fever cases admitted to public hospitals in Hong Kong

in 2003 were sporadic.

Seasonality

In Hong Kong, dengue cases were reported all year round. Figure 2 demonstrates the seasonal variation of cases, with a peak from July to September.

Country of origin for infection

Among the 116 imported cases, 106 (91%) were acquired in South-East Asian countries (Indonesia, Thailand, the Philippines, Vietnam, Singapore, Malaysia, Cambodia, Macau, and the Pacific Islands), eight (7%) originated from South Asia (India, Pakistan, Bangladesh, Sri-Lanka, and Nepal), and one (1%)

from Pitcairn island. Data for one case could not be determined as the patient had recently travelled to more than one country where the infection was endemic.

Patient demographics

The median age of the patients was 38 (range, 5-72) years and the female-to-male ratio was 1:1.2; five (4%) were paediatric patients (aged under 16 years); 114 (90%) were Hong Kong residents. A small proportion of the patients were migrant workers or tourists (4% and 5%, respectively). Among the Hong Kong residents, 86 (75%) were Chinese, 11 (10%) were from other Asian nations (Indonesia, the Philippines, Myanmar, Thailand), three (3%) were White and two (2%) belonged to the Pakistani/Nepalese group. Data on the origin of the remaining 12 patients were missing.

Serotype prevalence

Laboratory data on reverse-transcription PCR serotyping were available since 2002 and the serotypes of the corresponding 56 cases are shown in Figure 3.

All four serotypes, DEN-1, DEN-2, DEN-3 and DEN-4 were present among imported cases; while only DEN-1 (n=6) and DEN-2 (n=1) were present in local cases. Overall, DEN-1 was the most prevalent dengue serotype, responsible for nearly half (48%, 27/56) of all cases, followed by DEN-2 which accounted for about one quarter (23%, 13/56).

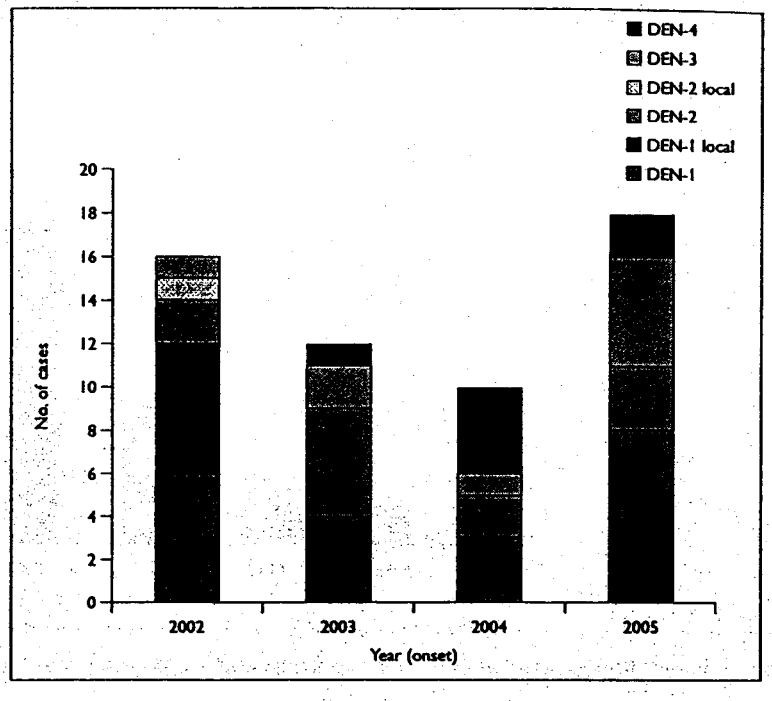


FIG 3. Distribution of serotypes among the dengue fever cases identified from 2002 to 2005

DEN-1 denotes dengue virus type 1, DEN-2 dengue virus type 2, DEN-3 dengue virus type 3, and DEN-4 dengue virus type 4

Clinical presentations and outcome

Approximately 98% (122/124) of patients presented with fever; the mean value for the highest temperature being 38.2°C (standard deviation, 1.0°C) [Table 1]. The second commonest presenting symptom was myalgia, 83% (75/90). Two thirds of patients had headache, fatigue, and skin rashes. One third of the patients (24/71) complained of retro-orbital pain. The chief presenting complaints in more than one third of the patients were gastro-intestinal (nausea, vomiting and/or diarrhoea) or upper respiratory tract (dry cough and/or sore throat) or both. Over one quarter of patients (28/108, 26%) complained of abdominal pain, and one complained of blurred vision. Except for petechiae which were present in 45% (47/105) of the patients, other spontaneous bleeding was uncommon. Maculopapular skin rash was the commonest physical finding; in 70% of those with a rash it occurred predominately on the trunk. Lymphadenopathy was uncommon, which was only elicited in 16% of the patients. No patient demonstrated biphasic fever. Only one patient had clinical and radiological features of plasma leakage (pleural effusion), and was confirmed to be due to DHF as the final diagnosis. The mean duration of hospitalisation for these patients was 6 days, and there was no mortality.

Laboratory findings

Thrombocytopenia was the most common haematological finding, which affected 107 (86%) of the 124 patients with available platelet counts (Table 1). The mean value of the lowest platelet counts was 64 x 10^9/L. Among those with available results, neutropenia, atypical lymphocytes, and lymphopenia were present in 78%, 75%, and 69% of the patients respectively; half had prolonged activated partial thromboplastin time values. Corresponding proportions with deranged liver function tests and hypoalbuminaemia are also shown in Table 1. Mean values for aspartate aminotransferase and alanine aminotransferase were 212 IU/L and 169 IU/L, respectively.

Clinical differential diagnosis

Dengue infection was included as an initial clinical differential diagnosis in only 29% of the patients. Other differential diagnoses included: viral infection, upper respiratory tract infection, gastroenteritis, typhoid fever, chest infection, malaria, scarlet fever, scrub typhus, influenza, and fever for investigation.

TABLE 1. Recorded clinical symptoms, physical and laboratory findings of dengue cases

Symptoms/findings	No. of patients (%)	Remarks (reference range for laboratory tests)
Clinical symptoms		
Fever	122/124 (98)	
Myalgia	75/90 (83)	
Headache	68/105 (65)	
Skin rash	72/121 (60)	
Fatigue	50/85 (59)	
Dizziness	20/44 (45)	
Retro-orbital pain	24/71 (34)	
Gastro-intestinal tract (nausea, vomiting, and/or diarrhoea)	39/112 (35)	
Upper respiratory tract (non-productive cough, sore throat)	32/112 (29)	
Bleeding manifestations		
Epistaxis	7/67 (10)	
Gum bleeding	8/66 (12)	
Haematemesis	1/65 (2)	Dengue haemorrhagic fever
Tarry stool	1/69 (1)	Dengue haemorrhagic fever
Petechiae	47/105 (45)	
Clinical signs		
Skin rash	86/124 (69)	
Lymphadenopathy	19/116 (16)	
Laboratory findings		
Thrombocytopenia	107/124 (86)	Platelets: 145-370 x 10^9/L
Lymphopenia	79/114 (69)	Lymphocytes: 1.0-3.1 x 10^9/L
Neutropenia	89/114 (78)	Neutrophils: 1.7-5.8 x 10^9/L
Atypical lymphocytes	92/123 (75)	
Prolonged activated partial thromboplastin time	49/97 (51)	Activated partial thromboplastin time: 27.5-40.5 sec
Elevated aspartate aminotransferase	29/32 (91)	Aspartate aminotransferase: <38 IU/L
Elevated alanine aminotransferase	98/123 (80)	Alanine aminotransferase: 3-36 IU/L
Hypoalbuminaemia	34/123 (28)	Albumin: 35-52 g/L

Comparison of epidemiological, clinical, and laboratory findings among the four dengue virus serotypes

There were no statistically significant differences in terms of disease severity between the four virus types, patient gender, age and duration of hospitalisation, headache, myalgia, arthralgia, retro-orbital pain, skin rash, fatigue, gastro-intestinal and respiratory symptoms (Table 2). The percentages of patients with bleeding tendencies were 50%, 67%, 63%, and 33% for DEN-1, DEN-2, DEN-3, and DEN-4 virus type infections, respectively. Further analysis of the haemorrhagic manifestations was conducted by categorisation into epistaxis, gum bleeding, haematuria, and petechiae; 75% of these patients exhibited petechiae only, with no statistically significant difference between virus types (P=0.58). Nor was there any statistically significant difference between patients having different virus subtype infections for laboratory variables, except that the lowest lymphocyte counts of patients infected by serotype 3 was lower than the other serotypes (P=0.004).

Dengue haemorrhagic fever

Of the 126 patients under study, three (2%) were classified as DHF; all were imported from South-East Asian countries, and none could recall a previous history of dengue infection. Their demographic, clinical, and laboratory findings are shown in Table 3. They all received intravenous fluid replacement and platelet transfusions, recovered uneventfully without progression to dengue shock syndrome, and were discharged on day 6 or day 7 after hospital admission. Although these three patients did not recall prior infection, in one it was likely, as evidenced by respective acute and convalescence antibody titres.

Discussion

This is a comprehensive review of dengue fever patients admitted to Hong Kong public hospitals over the past 8 years. Epidemiological data showed that more than 70% of the patients were local Chinese residents with a travel history to neighbouring South-East Asian countries, where dengue fever is more endemic.[6] The most prevalent serotype was DEN-1, followed by DEN-2, DEN-3, and DEN-4, which was consistent with the serotype patterns in the countries from which such infections were imported.[7,8] The outbreak in Ma Wan was the first local one in Hong Kong; only DEN-1 and DEN-2 virus subtypes were encountered in local patients during 2002 and 2003.

Seasonal variations in dengue infections should be interpreted with cautions. Dengue fever is a travel-related arthropod-borne viral disease in Hong Kong; disease activity is closely related to and depends on the seasonal and weather conditions of countries from which the virus is imported. It is difficult to determine the seasonal patterns of dengue fever acquired locally based on the few reported cases. Monthly ovitrap surveillance in Hong Kong showed that the density of *Aedes albopictus* increases from April and peaks in June.[9] It is important to alert the public to keep vigilance against this mosquito-borne viral disease during this peak period.

We report here the first blood transfusion–transmitted dengue in the literature. The patient was a 76-year-old woman, with a history of hypertension and bronchiectasis. She was admitted in 2002 because of progressive malaise. Blood tests revealed