Dengue virus in blood donations, Puerto Rico, 2005

Hamish Mohammed, Jeffrey M. Linnen, Jorge L. Muñoz-Jordán, Kay Tomashek, Gregory Foster, Amy S. Broulik, Lyle Petersen, and Susan L. Stramer

BACKGROUND: A single instance of transfusion-transmitted dengue infection has been reported. The high incidence of dengue in endemic countries, the high proportion of asymptomatic infection, and the median 5-day viremia, however, suggest that transfusion-associated dengue transmission may be more widespread than documented.

STUDY DESIGN AND METHODS: The prevalence of dengue virus (DENV) RNA was determined in all blood donations to the American Red Cross in Puerto Rico from September 20 to December 4, 2005, using a specific type of nucleic acid amplification test called transcription-mediated amplification (TMA). TMA-positive donations were defined as those having two repeatedly reactive TMA results. TMA-positive donations were tested by enzyme-linked immunosorbent assay for immunoglobulin M (IgM) antibodies, by reverse transcription–polymerase chain reaction (RT-PCR), and by viral culture.

RESULTS: Twelve (0.07%) of 16,521 blood donations tested were TMA-positive. Four were positive by RT-PCR (DENV serotypes 2 and 3). Virus was cultured from 3 of 4 RT-PCR–positive donations. One of the 12 TMA-positive donations was IgM-positive. Only 5 donations remained TMA-positive when diluted 1:16, as is done for routine minipool screening for other transfusion-transmissible viral infections (hepatitis C, human immunodeficiency, West Nile viruses [WNVs]).

CONCLUSION: Nearly 1 in 1000 blood donations contained DENV RNA, and virus could be cultured from TMA-positive donations, suggesting a transfusion transmission risk similar to that which existed in the United States for WNV before universal donation screening. Similar to WNV, IgM antibody screening is likely to be ineffective, and some potentially infectious donations will be missed by minipool screening. Transfusion transmission should be considered in patients with dengue after blood transfusion.

Dengue virus (DENV) is a mosquito-borne flavivirus transmitted by the bite of an infected *Aedes* spp. mosquito. Infection by each of the antigenically distinct serotypes (DENV-1, -2, -3, and -4) confers lifelong serotype-specific immunity. Subsequent infection with another serotype is possible because immunity to heterologous serotypes is short-lived. Most (53%-87%) dengue infections are asymptomatic or mildly symptomatic.[1-3] Dengue infection is characterized by a median 5-day viremia, and in clinically apparent infections, symptom onset occurs 1 day after onset of viremia.[4,5] The clinical spectrum of dengue infection ranges from dengue fever to dengue hemorrhagic fever, dengue shock syndrome, and death. Primary dengue infections often present with features of classic dengue fever including acute onset of fever, arthralgia, myalgia, retroorbital pain, headache, and rash. Subsequent infection with a second dengue serotype increases the risk of developing dengue hemorrhagic fever, which is characterized by fever, thrombocytopenia (platelet count $\leq 100 \times 10^9$/L), hemorrhagic manifestations, and evidence

ABBREVIATIONS: ARC = American Red Cross; DENV = dengue virus; IC = internal control; IR = initially reactive; S/CO ratio = signal-to-cutoff ratio; TMA = transcription-mediated amplification; WNV = West Nile virus.

From the Dengue Branch, Division of Vector-Borne Infectious Disease, Centers for Disease Control and Prevention, San Juan, Puerto Rico; American Red Cross, Gaithersburg, Maryland; Gen-Probe, Inc., San Diego, California; and the Division of Vector-Borne Infectious Disease, Centers for Disease Control and Prevention, Fort Collins, Colorado.

Address reprint requests to: Hamish Mohammed, PhD, Dengue Branch, Division of Vector-Borne Infectious Diseases, National Center for Zoonotic, Vector-Borne, and Enteric Diseases, Centers for Disease Control and Prevention, 1324 Calle Cañada, San Juan, PR 00920; e-mail: hmohammed@cdc.gov.

Conflict of interest: JML and ASB are employees of Gen-Probe, Inc. and are owners of equity stock options in Gen-Probe.

Received for publication December 13, 2007; revision received March 31, 2008, and accepted March 31, 2008.

doi: 10.1111/j.1537-2995.2008.01771.x

TRANSFUSION 2008;48:1348-1354.

of increased vascular permeability and plasma leakage.[6,7] With timely supportive care, dengue hemorrhagic fever case-fatality rates can be reduced to less than 1 percent.[8,9]

The principal dengue vector is *Aedes aegypti*. It is found throughout the tropics and subtropics and in limited areas of some states in the southeastern United States. *Aedes albopictus* is also a competent vector for dengue and has been implicated previously in dengue outbreaks.[10] Although it has not been detected in Puerto Rico, *A. albopictus* exists in some parts of the Americas including more than 20 states in the eastern half of the United States.[11] Autochthonous dengue transmission does sporadically occur in southern Texas along the United States-Mexico border, with the most recent outbreak occurring in the contiguous border towns of Brownsville, Texas, and Matamoros, Tamaulipas (Mexico).[12,13] This suggests the endemicity of dengue in South Texas and the risk of reemergence of dengue in states that border Mexico as well as in southeastern states with competent vector(s) and subtropical climates. Research, however, has found that differences in housing (e.g., use of air conditioning and screens) and lifestyle may prevent this from happening.[12,13]

Although few reports document DENV transmission through receipt of infected blood,[14] tissues,[15] or organs,[16] transfusion-associated dengue transmission may be more common than previously recognized. The high proportion of asymptomatic infections, the median 5-day period of detectable viremia, and the high incidence, especially during outbreaks, suggest that a substantial number of donors could be viremic at the time of donation. In addition, nosocomial transmission of DENV via needle-stick injury[17-21] further indicates the transmissibility of DENV by infected blood. Viremic individuals may unknowingly donate blood before symptom onset or if they remain asymptomatic. West Nile virus (WNV), a related mosquito-borne flavivirus, may provide a useful model for assessing transfusion-associated DENV transmission. Transfusion transmission of WNV is well documented, and all blood donations in the United States are screened using WNV-specific nucleic acid amplification tests (NATs).[22,23]

Dengue was first identified in Puerto Rico in 1963 and is now endemic year-round with occasional islandwide outbreaks. A mean of 5446 (range, 2416-10,048) suspected cases were reported annually during the nonoutbreak years from 1990 to 2004, whereas 6039 cases were reported in 2005 (incidences of 151 versus 159 per 100,000 population/year). Approximately 77,000 blood donations are collected annually by the American Red Cross (ARC) collection centers and blood donation drive sites in Puerto Rico. These donations are used in the continental United States, Puerto Rico, and elsewhere in the Caribbean. To assess the potential for transfusion-associated dengue infection in Puerto Rico, we tested all blood donations to the ARC for dengue viral nucleic acid using a recently developed dengue-specific NAT during an 11-week period of seasonally heightened dengue activity in 2005.

MATERIALS AND METHODS

We analyzed demographic data collected from blood donors and plasma specimens from all blood donations to ARC blood collection centers and blood drives in Puerto Rico from September 20 to December 4, 2005. This study period commenced 2 weeks after the peak of seasonally heightened dengue activity in Puerto Rico (Fig. 1). Plasma specimens containing ethylenediaminetetraacetate as an anticoagulant (BD Vacutainer PPT plasma preparation tubes, BD, Franklin Lakes, NJ) from all blood donations during this study period were retained in a repository at the ARC facility in Gaithersburg, Maryland.

All specimens were first screened for the presence of DENV RNA using a DENV-specific NAT developed by Gen-Probe, Inc. (San Diego, CA) that uses transcription-mediated amplification (TMA). Specimens were tested by TMA at Gen-Probe by trained ARC staff. All initially reactive (IR) specimens were retested and TMA-positive

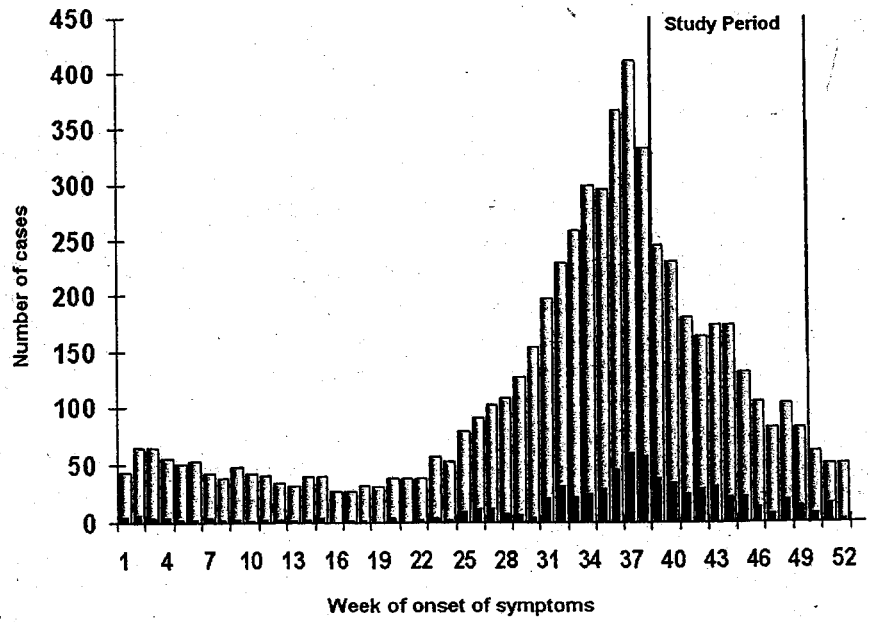

Fig. 1. Number of suspected* (□) and confirmed† (■) dengue cases by week of symptom onset, Puerto Rico, 2005. *Suspected = reported case of dengue with a clinical suspicion of dengue. †Confirmed = laboratory-confirmed (by serology or virology) case of dengue.

specimens were those that were repeatedly reactive; all others were considered to be TMA-negative. Both initial and repeat TMA screening were performed using individual specimens. All IR specimens were sent to the CDC's Dengue Branch Laboratory in San Juan, Puerto Rico, for supplemental testing by reverse transcription–polymerase chain reaction (RT-PCR).[24] Testing of donations was unlinked to donor personal identifiers; thus, subsequent contact with donors or recipients was not possible. Deidentified data from blood donation records were used in the statistical analysis (described below). The data were stored on a single password-protected terminal at the CDC, and no attempt was made to trace the donors. The study protocol was approved by the Institutional Review Board of the ARC.

TMA

Testing was performed using a prototype dengue TMA assay on a fully automated system for NAT blood screen (Procleix Tigris system, Chiron Corp., Emeryville, CA). The assay uses the same chemistry as other human immunodeficiency virus-1/hepatitis C virus and WNV assays (Procleix and Ultrio, respectively, Chiron Corp.)[25-27] and targets sequences that are conserved across all four serotypes. Thus the assay used is capable of detecting all four dengue serotypes. TMA is an isothermal RNA transcription amplification system using bacteriophage T7 RNA polymerase and Moloney murine leukemia virus reverse transcriptase (MMLV RT) to produce RNA amplicons via DNA intermediates. Viral lysis and magnetic-based target capture of viral RNA are followed by amplification and detection with the use of chemiluminescent probes.[26] This technique is able to detect 3.4 West Nile viral copies per mL at a 50 percent detection rate.[28] The analytical sensitivity of the DENV TMA assay used in this study is very similar, with 50 percent detection at 3.5 viral RNA copies per mL and a sample volume of 0.50 mL.[29] Assay results were reported in relative light units, which were used to derive signal-to-cutoff (S/CO) ratios. Cutoff values for the Dengue TMA assay internal control (IC) and analyte signals were calculated using the same formulae used for the Procleix WNV Assay.[30] A sample was considered reactive if the analyte S/CO ratio was at least 1.0, nonreactive if the analyte S/CO ratio was less than 1.0 and the IC signal was above the IC cutoff, and invalid if the analyte S/CO ratio value was less than 1.0 and the IC signal was below the IC cutoff.

Supplemental testing

All TMA-positive specimens were retested at a 1:16 dilution in plasma screened negative for all infectious disease markers including dengue RNA at Gen-Probe to determine the efficacy of testing blood donations by minipooled methods. The TMA-positive and IR specimens were tested using a real-time RT-PCR assay for the detection of NS5 gene sequence (TaqMan, Applied Biosystems, Foster City, CA).[24] This RT-PCR test is multiplexed and detects the four dengue serotypes in one reaction. It can also be used to quantitatively measure viral RNA in blood specimens with a sensitivity of approximately 1×10^3 to 5×10^3 viral RNA copies per mL. The sample volume is 20 μL derived from a 100-μL RNA extract obtained from a 0.24-mL serum specimen. All TMA-positive and IR specimens were also tested for the presence of immunoglobulin M (IgM) and immunoglobulin G (IgG) antibody using IgM MAC–enzyme-linked immunosorbent assay (ELISA) and IgG ELISA, respectively.[31,32] Virus was isolated on C6/36 cells and by mosquito isolation.[33,34]

Statistical analysis

A trend analysis using simple linear regression was performed to determine if there was a change in the number of blood donations collected during the study period. The prevalence of DENV RNA was determined by dividing the number of TMA-positive donations by the number of blood donations collected during the study period. Information about donor characteristics (see Table 1) was obtained from the ARC's electronic donor database and included date of collection, gender, date of birth, zip code of residence, zip code of donation site, donation status (first-time donor or repeat donor), phlebotomy procedure (whole blood, plateletpheresis, or leukapheresis), and donation type (allogeneic, directed, or autologous). Both donor residence and donation site were recoded into the three regions of Puerto Rico set by the United States Postal Service (San Juan Metropolitan Area, west, and east) and then treated as binary variables in the analyses (i.e., San Juan metropolitan area versus other). Age was stratified by its median and considered as a binary categorical variable in analyses.

Differences in TMA positivity by donor characteristics were assessed by the Fisher's exact test and exact logistic regression. Potential covariates identified for inclusion in the final multivariable model included covariates with a p value less than 0.20 on bivariate analysis. Age was added to the model a priori given its association with dengue infection.[2] All comparisons were made with the use of a two-tailed test, and a Type I error rate of 0.05 was used to assess significance.

RESULTS

A total of 16,521 blood donations were collected during the 11-week study period (mean, 1502 donations per week [range, 281-1864] without a significant trend in donation frequency). Twelve donations (0.73 per 1000 donations) were TMA-positive, with two or less identified per week. Eleven of these 12 donations were whole

blood-collections, while the other was a plateletpheresis from an O-donor. Donor and donation characteristics were similar among the TMA-positive and -negative donations ($p > 0.05$ for all variables, Fisher's exact test). In a multivariable model adjusted for age, donors with residence in the San Juan metropolitan area were approximately three times more likely than donors residing outside of the metropolitan area to be TMA-positive (adjusted odds ratio, 3.0; 95% confidence interval, 0.9-10.1).

The five blood donations that had the highest S/CO ratios on initial TMA testing were the only specimens to be TMA-reactive at a dilution of 1:16 (Table 2). Four of these five specimens were positive by RT-PCR and had quantifiable viral loads ranging from 2×10^3 to 8×10^7 viral RNA copies per mL. Three were identified as DENV-2 and the other as DENV-3. DENV was cultured from three of the four specimens, two by mosquito inoculation and one in cell culture. DENV-2 and DENV-3 were the predominant serotypes in circulation in Puerto Rico in 2005.

Serologic testing of the 12 TMA-positive blood donations revealed that only 1 was IgM-positive and 9 were IgG-positive by ELISA (Table 2). The lack of IgG antibody titers in Specimens 1, 4, and 8 indicates no previous dengue infections in these patients. The presence of IgG antibodies in the absence of IgM antibodies could reflect evidence of previous infections in Specimens 2, 3, 5, 7, 9, 10, 11, and 12, and IgG titers equal or greater than 1:163,840 in Specimens 3, 7, 9, and 10 indicate a recent or current secondary infection in those patients.[32] The presence of IgG in the sole donor with IgM antibodies (Specimen 6) could similarly be reflective of recent or current infection.

Other than the 12 TMA-positive specimens, there were an additional three IR specimens with S/CO ratios on initial testing of 1.00, 1.03, and 11.58 and on repeat testing of 0.92, 0.40, and 0.07, respectively. All were negative on PCR, IgM MAC-ELISA, and virus recovery. They were, however, positive on IgG ELISA. In the WNV TMA assay, an S/CO ratio of greater than or equal to 17 has a positive predictive value for confirmation of 95 percent (ARC data, unpublished); it is likely that this relationship is the same for DENV TMA.

TABLE 1. Characteristics of all and TMA-positive blood donors in Puerto Rico, September 20 to December 5, 2005*

Characteristic	All donors (n = 16,521)	TMA-positive donors (n = 12)
Age (years)	37.0 (13-85)	36.5 (16-65)
Male	10,654 (64.5)	8 (67)
Donation status		
First-time donor	5,056 (30.6)	5 (42)
Repeat donor	11,465 (69.4)	7 (58)
Region of residence		
San Juan Metropolitan Area	6,631 (40.1)	8 (67)
East	5,182 (31.4)	3 (25)
West	4,706 (28.5)	1 (8)
Phlebotomy procedure		
Whole blood	15,838 (95.9)	11 (92)
Plateletpheresis	627 (3.8)	1 (8)
Plateletpheresis/RBC pheresis	48 (0.3)	0 (0)
Double RBC pheresis	7 (0.0)	0 (0)
Leukapheresis	1 (0.0)	0 (0)
Donation type		
Allogeneic	16,400 (99.3)	12 (100)
Directed	67 (0.4)	0 (0)
Autologous	54 (0.3)	0 (0)
Region of donation site		
San Juan Metropolitan Area	8,984 (54.4)	8 (67)
East	3,870 (23.4)	4 (33)
West	3,667 (22.2)	0 (0)

* Data are reported as median (range) or number (%).

TABLE 2. Results of supplementary testing of TMA IR specimens (n = 12)

TMA test Gen-Probe (S/CO ratio)*				Supplementary testing CDC dengue branch					
Specimen	Initial test	Second test	1:16	PCR†	Number viral RNA/mL	IgM‡	IgG	Cell culture	Mosquito inoculation
1	31.96	26.99	27.73	D2	7.14×10^3	0.229	Negative	Negative	D2
2	30.31	31.28	28.78	D3	8.12×10^7	0.337	1:10,240	Negative	D3
3	29.22	27.86	27.12	D2	7.74×10^5	0.409	1:163,840	D2	Negative
4	29.17	24.84	22.92	D2	2.0×10^3	0.229	Negative	Negative	Negative
5	23.89	20.59	8.54	Negative	Undetected	0.469	1:2,560	Negative	Negative
6	21.22	5.28	0.21	Negative	Undetected	8.870	1:160	Negative	Negative
7	17.78	23.10	0.15	Negative	Undetected	0.409	1:655,360	Negative	Negative
8	17.41	18.44	0.31	Negative	Undetected	0.198	Negative	Negative	Negative
9	17.24	21.05	0.33	Negative	Undetected	1.540	1:163,840	Negative	Negative
10	5.97	7.73	0.15	Negative	Undetected	0.440	1:655,360	Negative	Negative
11	4.08	4.15	0.13	Negative	Undetected	0.368	1:10,240	Negative	Negative
12	1.53	5.56	0.60	Negative	Undetected	0.270	1:2,560	Negative	Negative

* S/CO ≥ 1 considered to be reactive.
† D2 = DENV-2, D3 = DENV-3.
‡ >2.000 considered positive.

Nine of the 12 repeat-reactive samples had S/CO values in one or both tests of 17 or greater.

DISCUSSION

This study, and a similar one recently conducted using donations in Honduras, Brazil, and Australia,[29] are the first to document the presence of dengue viral nucleic acid in blood donations. In Puerto Rico, nearly 1 in 1000 donations was positive for the presence of dengue viral nucleic acid by TMA. Furthermore, live virus was recovered from three of the 12 TMA-positive donations, indicating that at least these 3 were capable of transmitting infection to recipients. The prevalence of dengue viral nucleic acid in blood donations in this study was similar to that estimated for WNV in the areas experiencing outbreaks in the continental United States in 2002[35] before universal screening using minipool NAT was implemented in July 2003.[22] Assuming an annual prevalence rate of 0.73 per 1000 (as found in this study) and that each donation is made into a mean of 1.45 transfusable components,[36] there may be as many as 56 potentially viremic donations and 81 components generated from the approximately 77,000 blood donations collected annually by the ARC in Puerto Rico. Dengue incidence is highly seasonal and varies considerably from year to year,[37,38] however, so the prevalence of potentially viremic donors could be considerably higher or lower than this figure at any given time. Furthermore, the three IR specimens lacking reproducible results in repeat TMA testing may have been true-positive specimens but with lower viral loads. If the case, this would underestimate the true prevalence of TMA positivity.

The unlinked study design did not permit contact with the recipients of the TMA-positive donations to assess whether transmission occurred. Nevertheless, virus was cultured from three donations and the viral loads of the four RT-PCR–positive donations indicate that their transfusion would have resulted in inocula orders of magnitude greater than the amount of virus secreted in the saliva of *Aedes* mosquitoes, documented to be as low as 10^2 viral particles per secretion.[39] The RT-PCR assay used in this study had lower sensitivity than the TMA assay, and it was not possible to assess the viral load of the RT-PCR–negative specimens.

Our results indicate the feasibility of NAT as a screening strategy for DENV, as has been successfully used for WNV. Of concern, we found that simulated minipool NAT (dilution 1:16) would not have detected the majority (7 of 12, or 58%) of the TMA-positive specimens; however, the experience with WNV suggests that not all of these donations may be infectious. Approximately 30 percent of WNV NAT–positive donations have viral loads below the limits of detection by minipool NAT and can only be detected by screening of individual donations.[23,40] Although WNV has been transmitted from transfusions detectable only by individual unit screening and with an estimated level of viremia as low as 0.06 plaque-forming units (PFUs) per mL (1 PFU is approximately 400 viral copies),[41] most donations only detectable by individual unit screening had IgM and IgG antibodies and were likely not infectious given the fact that nearly all WNV transfusion transmissions have occurred from antibody-negative donations.[22,25,42] Unfortunately, this same marker of infectivity is not applicable to dengue because of the high prevalence of preexisting, cross-reactive dengue antibodies in the population and the complex and variable serologic response after secondary dengue infection.[32,43]

The global incidence of dengue has risen more than 30-fold in the past 50 years. In areas where dengue is endemic, however, transfusion transmission of the agent is rarely investigated for many reasons, including the fact that this mode of transmission is difficult to prove against a background of endemic dengue. In such cases, the distinction between a recipient infection via mosquito-borne transmission as opposed to transfusion transmission may be too complex to distinguish. Furthermore, many dengue-endemic countries lack hemovigilance systems with sufficient resources to investigate cases of recipient infection that are potentially related to transfusion of blood components. Finally, sophisticated laboratory testing may not be readily available in many dengue-endemic countries and such testing is required to distinguish dengue from other arboviral infections as well as distinguishing current dengue infection from prior infections.

In contrast, when WNV entered the United States, it was against a background of a naïve population. This permitted the laboratory linkage of multiple transfusion recipients with WNV infection to a single infected donor within several clusters of WNV cases. Infectious virus and/or viral RNA could also be recovered from retrieved cocomponent plasma units; in these cases, WNV was readily identified in the absence of competing arboviral infections. The transmissibility of WNV via blood transfusion has been established, and our findings documenting the presence of DENV RNA in the Puerto Rican blood supply, at a level comparable to that which triggered screening of the US blood supply for WNV in 2003, highlight the risks to transfusion safety posed by emerging diseases such as the vector-borne flaviviruses. Further evaluation is required to assess the risk of dengue transmission by TMA-positive donations and the cost and benefit of routine dengue screening in endemic regions.

ACKNOWLEDGMENTS

The authors acknowledge the following individuals for their contribution to this research: Mrs Dilia Magaly Borges and Mr Antonio de Vera (ARC-Puerto Rico) for providing data specific to the PR-region of ARC; Dr Mark Beatty (Pediatric Dengue Vaccine

Initiative) for assisting with protocol development; Mr Gilberto Santiago and Mr Mark Verduin (Dengue Branch, CDC) for laboratory support; Mr Michael Johansson (Dengue Branch, CDC) for statistical support; and Dr Ed Notari (ARC), Dr Mathew Kuehnert (Office of Blood, Organ, and other Tissue Safety (proposed), CDC), Mr Joshua Smith, Dr Kate McElroy, Dr D. Fermín Argüello, and Dr Wellington Sun (Dengue Branch, CDC) for their scientific input in preparing the manuscript.

REFERENCES

1. Endy TP, Chunsuttiwat S, Nisalak A, Libraty DH, Green S, Rothman AL, Vaughn AL, Ennis FA. Epidemiology of inapparent and symptomatic acute dengue virus infection: a prospective study of primary school children in Kamphaeng phet, Thailand. Am J Epidemiol 2002;156:40-51.
2. Rodriguez-Figueroa L, Rigau-Perez JG, Suarez EL, Reiter P. Risk factors for dengue infection during an outbreak in Yanes, Puerto Rico in 1991. Am J Trop Med Hyg 1995;52: 496-502.
3. Burke DS, Nisalak A, Johnson DE, Scott RM. A prospective study of dengue infections in Bangkok. Am J Trop Med Hyg 1988;38:172-80.
4. Vaughn DW, Green S, Kalayanarooj S, Innis BL, Nimmannitya S, Suntayakorn S, Endy TP, Raengsakulrach B, Rothman AL, Ennis FA, Nisalak A. Dengue viremia titer, antibody response pattern, and virus serotype correlate with disease severity. J Infect Dis 2000;181:2-9.
5. Nishiura H, Halstead SB. Natural history of dengue virus (DENV)-1 and DENV-4 infections: reanalysis of classic studies. J Infect Dis 2007;195:1007-13.
6. Halstead S. Dengue hemorrhagic fever—a public health problem and a field for research. Bull World Health Organ 1980;58:1-21.
7. Haemorrhagic D. Fever: diagnosis, treatment, prevention and control, 2nd ed. Geneva, Switzerland: World Health Organization; 1997.
8. Thang Hung N, Trong Lan N. Improvement of case management—a key factor to reduce case-fatality rate of dengue hemorrhagic fever in Southern Viet Nam. Dengue Bull 2003;27:144-8.
9. Chareonsook O, Foy H, Teeratkul A, Silarug N. Changing epidemiology of dengue hemorrhagic fever in Thailand. Epidemiol Infect 1999;122:161-6.
10. Effler PV, Pang L, Kitsutani P, Vorndam V, Nakata M, Ayers T, Elm J, Tom T, Reiter P, Rigau-Perez JG, Hayes JM, Mills K, Napier M, Clark GG, Gubler DJ; Hawaii Dengue Outbreak Investigation Team. Dengue fever, Hawaii, 2001-2002. Emerg Infect Dis 2005;11:742-9.
11. Moore C. Aedes albopictus in the United States: current status and prospects for further spread. J Am Mosq Control Assoc 1999;15:221-7.
12. Ramos M, Mohammed H, Zielinski-Gutierrez E, Hayden MH, Lopez JL, Fournier M, Trujillo AR, Burton R, Brunkard JM, Anaya-Lopez L, Banicki AA, Morales PK, Smith B, Munoz JL, Waterman SH, The Dengue Serosurvey Working Group. Epidemic dengue and dengue hemorrhagic fever at the Texas-Mexico Border: results of a household-based seroepidemiological survey, December 2005. Am J Trop Med Hyg 2008 Mar;78(3):364-9.
13. Reiter P, Lathrop S, Bunning M, Biggerstaff B, Singer D, Tiwari T, Baber L, Amador M, Thirion J, Hayes J, Seca C, Mendez J, Ramirez B, Robinson J, Rawlings J, Vorndam V, Waterman S, Gubler D, Clark G, Hayes E. Texas lifestyle limits transmission of dengue virus. Emerg Infect Dis 2003; 9:86-9.
14. Tsang C. Local dengue fever cases in 2002. Hong Kong, China: Public Health and Epidemiology Bulletin—Department of Health; 2002.
15. Rigau-Perez JG, Vorndam AV, Clark GG. The dengue and dengue hemorrhagic fever epidemic in Puerto Rico, 1994-1995. Am J Trop Med Hyg 2001;64:67-74.
16. Tan FL-S, Loh DL, Prabhakaran K. Dengue haemorrhagic fever after living donor renal transplantation. Nephrol Dial Transplant 2005;20:447-8.
17. Chen LN, Wilson ME. Nosocomial mucutaneous transmission and other routes of transmission. Emerg Infect Dis 2005;11:775.
18. De Wazieres B, Gil H, Vuitton D, Dupond J. Nosocomial transmission of dengue from a needle stick injury. Lancet 1998;351:498.
19. Nemes Z, Kiss G, Madarassi EP, Peterfi Z, Ferenczi E, Bakonyi T, Ternak G. Nosocomial transmission of dengue [letter]. Emerg Infect Dis 2004;10:1880-1.
20. Wagner D, de With K, Huzly D, Hufert F, Weidmann M, Breisinger S, Eppinger S, Kern WV, Bauer TM. Nosocomial acquisition of dengue. Emerg Infect Dis 2004;10:1872-3.
21. Hirsch J, Deschampes C, Lhuillier M. Metropolitan transmission of dengue by accidental inoculation at a hospital. Ann Intern Med 1990;141:629.
22. Pealer LN, Marfin AA, Petersen LR, Lanciotti RS, Page PL, Stramer SL, Stobierski MG, Signs K, Newman B, Kapoor H, Goodman JL, Chamberland ME; West Nile Virus Transmission Investigation Team. Transmission of West Nile virus through blood transfusion in the United States in 2002. N Engl J Med 2003;349:1236-45.
23. Stramer SL, Fang CT, Foster GA, Wagner AG, Brodsky JP, Dodd RY. West Nile virus among blood donors in the United States, 2003 and 2004. N Engl J Med 2005;353:451-9.
24. Chien LJ, Liao TL, Shu PY, Huang JH, Gubler DJ, Chang GJ. Development of real-time reverse transcriptase PCR assays to detect and serotype dengue viruses. J Clin Microbiol 2006;44:1295-304.
25. Busch MP, Tobler LH, Saldanha J, Caglioti S, Shyamala V, Linnen JM, Gallarda J, Phelps B, Smith RI, Drebot M, Kleinman SH. Analytical and clinical sensitivity of West Nile virus RNA screening and supplemental assays available in 2003. Transfusion 2005;45:492-9.
26. Giachetti C, Linnen JM, Kolk DP, Dockter J, Gillotte-Taylor K, Park M, Ho-Sing-Loy M, McCormick MK, Mimms LT,

McDonough SH. Highly sensitive multiplex assay for detection of human immunodeficiency virus type 1 and hepatitis C virus RNA. J Clin Microbiol 2002;40:2408-19.
27. McCormick MK, Dockter J, Linnen JM, Kolk D, Wu Y, Giachetti C. Evaluation of a new molecular assay for detection of human immunodeficiency virus type 1 RNA, hepatitis C virus RNA, and hepatitis B virus DNA. J Clin Virol 2006;36:166-76.
28. Linnen JM, Deras ML, Cline J, Wu W, Broulik AS, Cory RE, Knight JL, Cass MM, Collins CS, Giachetti C. Performance evaluation of the PROCLEIX West Nile virus assay on semi-automated and automated systems. J Med Virol 2007;79: 1422-30.
29. Linnen JM, Vinelli E, Sabino EC, Tobler LH, Hyland C, Lee TH, Kolk DP, Broulik AS, Collins CS, Lanciotti RS, Busch MP. Dengue viremia in blood donors from Honduras, Brazil and Australia. Transfusion DOI: 10.1111/j.1537-2995.2008.01772.x
30. Linnen JM, Deras ML, Cline J, Wu W, Broulik AS, Cory RE, Knight JL, Cass MM, Collins CS, Giachetti C. Performance evaluation of the PROCLEIX West Nile virus assay on semi-automated and automated systems. J Med Virol 2007;79: 1422-30.
31. Burke DS, Nisalak A, Ussery MA. Antibody capture immunoassay detection of Japanese encephalitis virus immunoglobulin M and G antibodies in cerebrospinal fluid. J Clin Microbiol 1982;16:1034-42.
32. Miagostovich MP, Nogueira RM, dos Santos FB, Schatzmayr HG, Araujo ES, Vorndam V. Evaluation of an IgG enzyme-linked immunosorbent assay for dengue diagnosis. J Clin Microbiol 1999;14:183-9.
33. Rosen L, Gubler D. The use of mosquitoes to detect and propagate dengue viruses. Am J Trop Med Hyg 1974;23: 1153-60.
34. Gubler DJ, Kuno G, Sather GE, Velez M, Oliver A. Mosquito cell cultures and specific monoclonal antibodies in surveillance for dengue viruses. Am J Trop Med Hyg 1984;33:158-65.
35. Biggerstaff BJ, Petersen LR. Estimated risk of transmission of the West Nile virus through blood transfusion in the US, 2002. Transfusion 2003;43:1007-17.
36. Marshall DA, Kleinman SH, Wong JB, AuBuchon JP, Grima DT, Kulin NA, Weinstein MC. Cost-effectiveness of nucleic acid test screening of volunteer blood donations for hepatitis B, hepatitis C and human immunodeficiency virus in the United States. Vox Sang 2004;86:28-40.
37. Rigau-Perez JG. Dengue activity in Puerto Rico during an interepidemic period (1995-1997). Am J Trop Med Hyg 2001;64:75-83.
38. Rigau-Perez JG. The reappearance of dengue-3 and a subsequent dengue-4 and dengue-1 epidemic in Puerto Rico in 1998. Am J Trop Med Hyg 2002;67:355-62.
39. Kraiselburd E, Gubler DJ, Kessler MJ. Quantity of dengue virus required to infect rhesus monkeys. Trans R Soc Trop Med Hyg 1985;79:248-51.
40. Busch MP, Caglioti S, Robertson EF, McAuley JD, Tobler LH, Kamel H, Linnen JM, Shyamala V, Tomasulo P, Kleinman SH. Screening the blood supply for West Nile virus RNA by nucleic acid amplification testing. N Engl J Med 2005;353:460-7.
41. Montgomery SP, Brown JA, Kuehnert M, Smith TL, Crall N, Lanciotti RS, Macedo de Oliveira A, Boo T, Marfin AA; 2003 West Nile Virus Transfusion-Associated Transmission Investigation Team. Transfusion-associated transmission of West Nile virus, United States 2003 through 2005. Transfusion 2006;46:2038-46.
42. Petersen LR, Epstein JS. Problem solved? West Nile virus and transfusion safety. N Engl J Med 2005;353:516-7.
43. Gubler DJ, Sather GE. Laboratory diagnosis of dengue and dengue hemorrhagic fever. In: Homma A, Cunha JF, editors. Proceedings of the international symposium on yellow fever and dengue. Rio de Janeiro, Brazil: Rio de Janeiro; 1988. p. 291-322.

医薬品
医薬部外品　研究報告　調査報告書
化粧品

別紙 3-7

識別番号・報告回数		回	報告日 年　月　日	第一報入手日 2008 年 6 月 13 日	新医薬品等の区分 該当なし	総合機構処理欄
一般的名称			研究報告の公表状況	A clinical trial of a whole-virus H5N1 vaccine derived from cell culture. Ehrlich, H.J. et al, New Engl. J. Med., 358, 2573-2584 (2008)	公表国 オーストリア	
販売名（企業名）						

研究報告の概要

本稿では，抗 H5N1 型鳥インフルエンザワクチンの安全性及び有効性を検討する無作為化用量漸増第 1・2 相試験の試験成績について記載した。Baxter Bioscience 社が同ワクチンを開発し，試験を実施した。当該ワクチンの主な特徴は，サルの腎臓細胞の培養株（ベロ細胞）で作成された自然発生するウイルス株 A/Vietnam/1203/2004 を利用していることである。ミョウバンアジュバントによる作用も検討し，ウイルス全体をワクチンとして使用した。有効性エンドポイントとして，ワクチンの（i）ヘマグルチニン阻害を生じさせる能力，（ii）中和抗体を誘発する能力，（iii）注射 21 日後にセロコンバージョンを生じさせる能力を検討した。各被験者に対し，それぞれ 3.75，7.5，15 又は 30 ug のヘマグルチニン抗原を含有するワクチンをアジュバントとともに，もしくは 7.5 又は 15 ug の抗原を含有するワクチンをアジュバントなしで 21 日の間隔をおいて 2 回投与した。免疫寛容は非常に良好に成立した。いずれのワクチン処方でも，注射部位の軽度疼痛（被験者の 9～27%）及び頭痛（被験者の 6～31%）が最も高頻度に報告された有害事象であった。有効性に関する限り，免疫応答はアジュバントなしの処方を投与した被験者において最も高い割合で認められたが，いずれの処方でも第 21 日目から第 42 日目で中和抗体の抗体価は同程度に増加した。さらに，ウイルス株 A/Indonesia/05/2005 及び A/Hong Kong/156/1997 に対する交差中和が認められた。再度，アジュバントなしの処方が最も高い免疫原性を示した。本試験では重要な用量反応性の関連が示されなかったことから，今後の開発にあたってアジュバントなしの 7.5 ug の処方が選択された。

使用上の注意記載状況・その他参考事項等

BYL-2008-0338

報告企業の意見	今後の対応
抗 H5N1 型鳥インフルエンザワクチンが利用可能となれば、パンデミックの発生及び拡大を防ぐために有効であろう。血漿由来製剤工程におけるウイルス除去は、インフルエンザウイルス除去に対しても有効と考えられる。弊社製品の製造工程に使用されている血漿分画成分は，製造工程中のウイルスバリデーションにおいて，インフルエンザウイルスと同様のエンベロープ RNA ウイルスである HIV（レトロウイルス）の不活化・除去能が確認されている。各成分の製造工程における不活化・除去能は以下のとおり。 • アルブミン・カッター及びコージネイト FS の製造工程培地に使用されているヒト血清アルブミン：17.8 log 以上 • プラスマネート・カッター，コージネイト FS 及びコージネイト FS バイオセットの製造工程培地に使用されている加熱ヒト血漿タンパク：15 log 以上 • コージネイト FS の製造工程に使用されているトランスフェリン：9.1 log 以上 • ベタフェロン皮下注，ゼヴァリン イットリウム (^{90}Y) 静注用セット及びゼヴァリン インジウム (^{111}I) 静注用セットの製造工程に使用されているヒト血清アルブミン：9.98 log 以上	現時点で新たな安全対策上の措置を講じる必要はないと考える。