

ORIGINAL ARTICLE

Transmission of West Nile Virus through Blood Transfusion in the United States in 2002

Lisa N. Pealer, Ph.D., Anthony A. Marfin, M.D., M.P.H.,
Lyle R. Petersen, M.D., M.P.H., Robert S. Lanciotti, Ph.D., Peter L. Page, M.D.,
Susan L. Stramer, Ph.D., Mary Grace Stobierski, D.V.M., M.P.H.,
Kimberly Signs, D.V.M., Bruce Newman, M.D., Hema Kapoor, M.D.,
Jesse L. Goodman, M.D., M.P.H., and Mary E. Chamberland, M.D., M.P.H.,
for the West Nile Virus Transmission Investigation Team*

ABSTRACT

BACKGROUND

During the 2002 West Nile virus epidemic in the United States, patients were identified whose West Nile virus illness was temporally associated with the receipt of transfused blood and blood components.

METHODS

Patients with laboratory evidence of recent West Nile virus infection within four weeks after receipt of a blood component from a donor with viremia were considered to have a confirmed transfusion-related infection. We interviewed the donors of these components, asking them whether they had had symptoms compatible with the presence of a viral illness before or after their donation; blood specimens retained from the time of donation and collected at follow-up were tested for West Nile virus.

RESULTS

Twenty-three patients were confirmed to have acquired West Nile virus through transfused leukoreduced and nonleukoreduced red cells, platelets, or fresh-frozen plasma. Of the 23 recipients, 10 (43 percent) were immunocompromised owing to transplantation or cancer and 8 (35 percent) were at least 70 years of age. Immunocompromised recipients tended to have longer incubation periods than nonimmunocompromised recipients and infected persons in mosquito-borne community outbreaks. Sixteen donors with evidence of viremia at donation were linked to the 23 infected recipients; of these donors, 9 reported viral symptoms before or after donation, 5 were asymptomatic, and 2 were lost to follow-up. Fever, new rash, and painful eyes were independently associated with being an implicated donor with viremia rather than a donor without viremia. All 16 donors were negative for West Nile virus-specific IgM antibody at donation.

CONCLUSIONS

Transfused red cells, platelets, and fresh-frozen plasma can transmit West Nile virus. Screening of potential donors with the use of nucleic acid-based assays for West Nile virus may reduce this risk.

From the Epidemic Intelligence Service, Division of Applied Public Health Training, Epidemiology Program Office (L.N.P.), and the Division of Viral and Rickettsial Diseases, National Center for Infectious Diseases (M.E.C.), Centers for Disease Control and Prevention (CDC), Atlanta; the Division of Vector-Borne Infectious Diseases, National Center for Infectious Diseases, CDC, Fort Collins, Colo. (A.A.M., L.R.P., R.S.L.); the American Red Cross Blood Services, Biomedical Headquarters, Washington, D.C. (P.L.P.); the American Red Cross Blood Services, Scientific Support Office, Gaithersburg, Md. (S.L.S.); the Michigan Department of Community Health (M.G.S., K.S.) and Bureau of Laboratories, Michigan Department of Community Health (H.K.), Lansing; the American Red Cross Blood Services, Southeastern Michigan, Detroit (B.N.); and the Food and Drug Administration, Center for Biologics Evaluation and Research, Rockville, Md. (J.L.G.). Address reprint requests to Dr. Pealer at the Centers for Disease Control and Prevention, 1600 Clifton Rd., D-18, Atlanta, GA 30333, or at lpealer@cdc.gov.

*Members of the West Nile Virus Transfusion Transmission Investigation Team are listed in the Appendix.

N Engl J Med 2003;349:1236-45.

Copyright © 2003 Massachusetts Medical Society.

WEST NILE VIRUS IS A MOSQUITO-borne flavivirus that is transmitted primarily among birds; humans serve as incidental hosts. In the United States, human infection with the virus was first recognized in 1999 in Queens, New York.^{1,2} By 2002, the known geographic range of West Nile virus had expanded to 44 states and the District of Columbia,³ and that same year, 4200 cases of West Nile virus–associated illness were reported in humans (Centers for Disease Control and Prevention [CDC]: unpublished data). This represents an increase by a factor of nearly 30 over the 149 cases reported in humans from 1999 through 2001.³ Although the transmission of West Nile virus by blood transfusion had not been reported before 2002, the findings of transient viremia after infection and a high proportion of asymptomatic or mildly symptomatic infections suggested that this route of transmission might be possible.⁴⁻⁶

In August 2002, in response to theoretical concern that West Nile virus could be transmitted through blood transfusions, the Food and Drug Administration and the CDC advised blood establishments and health departments to be alert for persons with West Nile virus infection who had donated blood the week before their illness began and for persons with unexplained fever-associated meningitis or encephalitis that developed after the receipt of a blood transfusion. In response to these messages, on August 30, 2002, a state health department notified the CDC of the first suspected case of transfusion-transmitted West Nile virus in a woman who had received blood and blood products related to an obstetrical procedure⁷; additional reports of West Nile virus infection among transfusion recipients quickly followed.⁸⁻¹⁰ During this period, an independent investigation of four patients in whom West Nile virus illness developed after they received solid organs from a single donor was initiated, which subsequently determined that the organ donor most likely acquired West Nile virus through a blood transfusion.¹¹ In this report, we summarize the findings of the investigations of transfusion recipients and blood donors.

METHODS

INVESTIGATIONS OF BLOOD DONORS AND TRANSFUSION RECIPIENTS

Investigations were conducted from August 28, 2002, to April 15, 2003. Patients with West Nile vi-

rus infection temporally associated with the receipt of blood transfusions were most often identified by physicians and reported initially to blood-collection agencies or state and local health departments.

The medical records of persons suspected of having become infected with West Nile virus through the receipt of transfused blood products were reviewed. Standardized abstraction forms were used to collect information on the clinical course of West Nile virus infection, underlying medical conditions, and blood components transfused within four weeks before the onset of West Nile virus–like illness. To confirm the diagnosis and to assess the evolution of laboratory markers of infection in these patients, we tested available pretransfusion and post-transfusion clinical specimens (e.g., cerebrospinal fluid, serum, and plasma) for West Nile virus RNA and IgM antibody.

Blood-collection agencies identified donors of blood components that had been transfused to West Nile virus–infected recipients in the four weeks before the onset of illness. Other blood components made from these donations (co-components), blood samples obtained from tubing attached to the original collection bag or the red-cell component (“retention segments”), and any other blood samples obtained at the time of donation were retrieved and tested for West Nile virus RNA and IgM antibody. If a donor was subsequently found to have evidence of West Nile viremia at the time of donation (Table 1), other recipients of co-components from these implicated donations were contacted for West Nile virus antibody testing and medical-record abstraction.

Blood-collection agencies conducted follow-up interviews of donors of blood components transfused to infected recipients with use of a standardized data-collection form. Donors were asked whether they had had symptoms compatible with the presence of a viral illness in the three weeks before or after their donation. A serum sample was collected for West Nile virus IgM antibody testing.

TESTING FOR WEST NILE VIRUS

RNA Assay

West Nile virus RNA was detected with the use of a quantitative, real-time, reverse-transcriptase–polymerase-chain-reaction (PCR) assay (TaqMan, Applied Biosystems). Samples were prepared with the use of previously described methods of RNA extraction (extraction volume of 200 μ l or less) and primer–probe designs (lower limit of detection,

Table 1. Case Classifications and Definitions of Transfusion-Transmitted West Nile Virus Infection.***Classification of cases**

A confirmed case required both of the following:

- Evidence of West Nile viremia in a donor
- Evidence of West Nile virus infection in a recipient of a component from a donor with viremia

A definite noncase required one or both of the following:

- Negative IgM antibody test on follow-up analysis of all donors of blood from which components were derived and given within 4 weeks before the onset of an illness compatible with West Nile virus infection
- Absence of laboratory evidence of West Nile virus infection in the recipient

An inconclusive case result required all of the following:

- Receipt of blood products
- Laboratory evidence of recent West Nile virus infection
- Failure to meet the definition for a confirmed case or a definite noncase

Definitions

Determination of viremia in a donor required one or more of the following:

- Isolation of West Nile virus from a sample obtained at the time of donation
- Positive PCR results on testing of sample obtained at the time of donation and evidence of IgM antibody seroconversion on follow-up testing
- Positive PCR results on testing of sample obtained at the time of donation, with no follow-up testing of the donor, and positive IgM antibody tests in two or more recipients of co-components from the implicated donation
- Equivocal PCR results on testing of sample obtained at the time of donation and documented IgM antibody seroconversion on follow-up testing of the donor and on testing of two or more of the recipients of co-components from the implicated donation

Determination of West Nile virus infection in the recipient of a component from a donor with viremia required at least one of the following:

- West Nile virus–associated illness within 4 weeks after the receipt of a component from a donor with viremia and laboratory evidence of recent West Nile virus infection
- Positive test for IgM antibody either without a history or with a possible history of illness compatible with West Nile virus infection and receipt of a co-component from a donor with a confirmed case

Determination of West Nile virus–associated illness in a recipient required the following:

- New onset of unexplained fever, meningitis, encephalitis, or acute flaccid paralysis (alone or in combination) after the transfusion of a component from a donor with viremia

Laboratory criteria for confirmed recent West Nile virus infection were as follows:†

- Isolation of West Nile virus from tissue, blood, or cerebrospinal fluid
- Detection of West Nile virus antigen by immunohistochemical staining or of West Nile virus genomic sequences in tissue, blood, or cerebrospinal fluid
- Detection of West Nile virus IgM antibodies in a cerebrospinal fluid sample obtained during the acute phase of illness by IgM-capture ELISA
- Recent seroconversion, with detection of West Nile virus IgM by IgM-capture ELISA

* PCR denotes polymerase chain reaction, and ELISA enzyme-linked immunosorbent assay.

† Data are from the Centers for Disease Control and Prevention.¹²

0.8 West Nile virus plaque-forming unit [pfu] per milliliter).¹³

If a donor had West Nile virus IgM antibody on follow-up testing and if the results of the standard-volume PCR assay were negative, samples obtained at the time of donation were assayed with the use of a modified TaqMan PCR assay. In this assay, RNA was extracted from a 500- μ l extraction volume, and 25 μ l of the RNA was used in the amplification reaction (the lower limit of detection for this high-volume extraction was 0.1 pfu per milliliter). Amplification and fluorescence were detected with the use of the iCycler iQ Real-Time PCR Detection System (Bio-Rad Laboratories); 45 cycles of amplification were performed according to the manufactur-

er's recommendations. In both assays, a positive result was defined as an increase in the fluorescent signal above a defined threshold value within 37 cycles for each of two different primer–probe sets and a change in fluorescence that was two or more times as great as the average signal of eight negative wells included in each assay run. In the negative wells, 5 μ l of RNase- and DNase-free water was substituted for 5 μ l of extracted RNA. An equivocal result was defined as a test that was positive for only one of the two primer–probe sets. All positive and equivocal PCR assays were repeated. Concentrations of West Nile virus in study samples were estimated by comparing the number of PCR cycles they required to surpass the threshold value with the

number of cycles required to do so by samples with known concentrations of virus. These known standards were obtained by making serial dilutions of West Nile virus seeded with human serum.

Viral Culture

When the sample was large enough, specimens positive for West Nile virus by PCR were tested for the virus by viral isolation on Vero cells. Positive results were defined by the occurrence of a cytopathic effect, which was confirmed by PCR assays.

Serologic Testing

Samples obtained at the time of donation and serum samples obtained at follow-up were assayed for West Nile virus-specific IgM antibodies with use of an IgM-capture enzyme-linked immunosorbent assay and confirmed with a plaque-reduction neutralizing-antibody test.^{13,14}

CLASSIFICATION OF CASES

A confirmed case of transfusion-transmitted West Nile virus infection required evidence of infection in the recipient of a component from a donor with confirmed viremia (Table 1). Reports lacking such data were classified as inconclusive or as not representing a case.

STATISTICAL ANALYSIS

Incubation periods were compared among the transfusion recipients with use of the t-test. The t-test was also used to compare the mean levels of viremia between symptomatic and asymptomatic donors. Fisher's exact test was used to compare symptoms of donors with evidence of viremia at donation with those of donors without viremia who were IgM-negative at follow-up (SAS, version 8.02, SAS Institute). Adjusted odds ratios for these symptoms were computed with the use of logistic regression, after adjustment for sex and age, to identify symptoms in the donor population that predicted West Nile virus infection. All comparisons were made with use of a two-tailed test, and P values of less than 0.05 were considered to indicate statistical significance.

RESULTS

TRANSFUSION RECIPIENTS

Of 61 patients identified as having possible transfusion-transmitted West Nile virus infection, 23 were confirmed to have transfusion-associated infection,

19 had inconclusive investigations, and 19 were determined not to have such an infection. Of the 19 persons with inconclusive results, 1 had received transfusions from a donor whose initial donation sample was positive for West Nile virus by PCR, but no follow-up sample could be obtained and no recipients of co-components were identified; 4 had a donor with West Nile virus IgM antibody, but the presence of viremia at the time of donation could not be proved, because samples were not available from the time of donation (in 2 donors) and were negative for West Nile virus by PCR (in 2 donors); and 14 had incomplete follow-up data for the donors. Of the 19 patients who were determined not to have an infection, 8 had no laboratory evidence of West Nile virus infection, 8 received components from donors without evidence of recent infection, 2 became ill before receiving transfusions, and West Nile virus illness developed in 1 more than four weeks after transfusion.

The 23 patients with confirmed infections ranged from 7 to 90 years of age (median, 48); 12 were female (Table 2). Ten of the 23 (43 percent) were immunocompromised as a result of bone marrow, stem-cell, or organ transplantation or hematologic or other advanced cancers, and 8 others (35 percent) were at least 70 years of age and had other medical and surgical problems. As shown in Table 2, 14 patients were initially assessed because West Nile virus-associated illness developed after the transfusion of blood components (Recipients 1 through 5, 7, 10, 12, 14, 16, 18, 21, 22, and 23), 7 because they had received a co-component from a donor with viremia who was linked to another recipient with West Nile virus illness (Recipients 6, 8, 9, 11, 13, 17, and 19), 1 (Recipient 15) because a donor notified the blood-collection agency of a post-donation diagnosis of West Nile fever, and 1 (Recipient 20) because of the possibility of transmission from transplanted organs.⁸ The seven recipients of co-components were IgM-positive on follow-up, but only one had a clearly defined illness related to West Nile virus infection.

Among the 15 recipients with West Nile virus-associated illness (13 with meningoencephalitis and 2 with fever), the illness began 2 to 21 days (median, 10) after transfusion of the implicated component (Table 2) (excluding Recipient 4, who received donations from two donors with viremia). Transplant recipients (Recipients 2, 7, 16, and 21) tended to have longer incubation periods (median, 13.5 days) than recipients without obvious immu-

Table 2. Demographic, Clinical, Laboratory, and Transfusion Data for 23 Patients with Confirmed Cases of Transfusion-Transmitted West Nile Virus in the United States, 2002.*

Recipient No.	Donor No.	Underlying Condition	Age (yr)/ Sex	Date of Transfusion	Type of Component	Days from Transfusion to Onset of Illness	Type of Illness	Vital Status of Recipient	Date Specimen Collected	Specimen Tested	Test Result†
1‡	1	Post partum	24/F	July 27	RBC	2	ME	Alive	Aug. 23	CSF	IgM+
2	2	Liver transplantation	47/M	Aug. 20	PLT	13	ME	Alive	Sept. 4 Sept. 5	Serum CSF	IgM+, PCR± IgM+, PCR-
3‡	2	Post partum	40/F	Sept. 3	RBC	10	ME	Alive	Sept. 27	Serum	IgM+, PCR-
4§	3	AML	12/F	Sept. 1	PLT	11	ME	Alive	Sept. 25	CSF	IgM+
4	4	—	—/—	Sept. 8	PLT	4	—	—	—	—	—
5‡	5	Lung cancer	60/M	Sept. 18	RBC	12	ME	Dead	Sept. 25 Sept. 30 Oct. 2¶	Serum Serum CSF	IgM-, PCR± IgM+, PCR- IgM+
6	5	Breast cancer	40/F	Oct. 6	FFP	3	ME	Alive	Oct. 15¶ Oct. 21	Serum Serum	PCR+, culture+ IgM+, PCR+
7	6	AML, bone marrow transplantation	59/M	Aug. 24	PLT	14	ME	Dead	Sept. 19 Sept. 24	CSF Serum	IgM-, PCR+, culture+ IgM-, PCR+, culture+
8	6	Aneurysm repair	75/F	Aug. 30	FFP	NA	—	Alive	Oct. 25	Serum	IgM+
9	6	AML, stem-cell transplantation	24/M	Sept. 10	RBC	NA	—	Alive	Oct. 29	Serum	IgM+
10	7	Rhabdomyosarcoma	7/M	Sept. 12	PLT	21	ME	Alive	Oct. 12	CSF	IgM+
11	7	Pneumonia, septic shock	73/F	Oct. 12	RBC	NA	—	Alive	Nov. 4	Serum	IgM+, PCR-
12‡	8	Coronary-artery bypass grafting	74/F	Sept. 3	RBC	Unknown	ME	Dead	Sept. 26 Oct. 9	Serum CSF	IgM+, PCR- IgM+, PCR-
13	8	Aortic-valve replacement	72/M	Aug. 22	PLT	NA	—	Alive	Dec. 13	Serum	IgM+
14	9	Coronary-artery bypass grafting	70/F	Sept. 23	PLT	8	ME	Alive	Oct. 8 Oct. 8	Serum CSF	IgM+ IgM+
15‡	10	AML, bone marrow transplantation	31/M	Sept. 9	RBC	NA	—	Dead	Sept. 24	Serum	IgM-, PCR+, culture+
16	11	Congenital bone marrow failure, stem-cell transplantation	7/M	Sept. 22	PLT	16	ME	Alive	Oct. 5 Oct. 14	Serum CSF	IgM-, PCR+ IgM+
17	11	Motor vehicle accident	33/M	Oct. 8	FFP	NA	—	Alive	Jan. 9	Serum	IgM+
18‡	12	Lumbar laminectomy	48/F	Oct. 12	RBC**	7	Fever	Alive	Oct. 24 Oct. 25	CSF Serum	IgM-, PCR- IgM+, PCR-
19‡	12	Femur-fracture repair	90/F	Oct. 23	RBC**	NA	—	Alive	Nov. 27	Serum	IgM+

20‡	13	Motor vehicle accident	17/F	July 31	RBC	NA	—	Dead	Aug. 1	Serum	IgM-, PCR+, culture+
21‡	14	Kidney transplantation	62/M	Oct. 4	RBC	10	ME	Dead	Oct. 15 Oct. 22	Serum Serum	IgM+, PCR+ IgM+, PCR±
22‡	15	Gastrointestinal bleeding	73/M	Aug. 22	RBC	14	Fever	Alive	Sept. 4	Serum	IgM+
23‡	16	Aplastic anemia	73/F	Oct. 5	RBC	10	ME	Dead	Oct. 23	Serum	IgM+

* RBC denotes red cells, ME West Nile virus meningoencephalitis, CSF cerebrospinal fluid, PLT platelets, PCR polymerase-chain-reaction assay, AML acute myelogenous leukemia, FFP fresh-frozen plasma, and NA not applicable.

† Plus signs indicate positive results, plus-minus signs equivocal results, and minus signs negative results.

‡ The recipient received leukoreduced red cells.

§ Investigation of this recipient identified two donors with PCR-positive donation samples, both with documented seroconversion at follow-up testing.

¶ Specimen testing was completed at the Wadsworth Centers at the New York State Department of Health.

|| The definite date of onset of West Nile virus illness could not be identified owing to the presence of multiple medical problems.

** Two red-cell units were produced from the donation of Donor 12.

nosuppressing conditions (Recipients 1, 3, 14, 18, and 22) (median, 8 days; $P=0.08$). Seven patients with confirmed cases of West Nile virus infection died, one of whom was the organ donor implicated in four cases of transplantation-associated infection (Recipient 20)⁸; the cause of death in the other six was unclear, because of underlying medical conditions.

Red cells (including 11 leukoreduced red cells), platelets, and fresh-frozen plasma were sources of exposure to West Nile virus (Table 2). The maximal interval from donation to transfusion (i.e., the observed period of survival of the virus) was 5 days for platelets, 33 days for red cells, and 44 days for fresh-frozen plasma. The 15 recipients with West Nile virus meningoencephalitis or fever had received blood components from a median of 18 donations (range, 2 to 274) within four weeks before the onset of illness.

PCR testing of stored serum was performed in the case of 12 recipients, and West Nile virus RNA was detected in 8. Among these eight recipients, seven had either a hematologic or solid-organ cancer or were receiving chemotherapy; West Nile virus RNA was detected a median of 15 days (range, 7 to 31) after transfusion of the implicated component. The eighth recipient was the organ donor (Recipient 20). Retrospective testing also showed that three recipients with positive PCR results (Recipients 7, 15, and 16) did not have West Nile virus antibody in serum samples collected 13 to 31 days after the implicated transfusion.

BLOOD DONORS

The 23 patients with confirmed infections were associated with 16 donors with viremia who had donated blood or blood components from July 22 to October 6 (Tables 2 and 3). Seven of these 16 donors were linked to two or three recipients with West Nile virus infection (Table 2). Recipient 4 received transfusions from two donors with viremia (Table 2). The 16 donations from the donors were made into 40 components; 14 were not transfused; the remaining 26 components were transfused into 25 recipients. Each of the 23 recipients who received blood from a donor with viremia and who could be tested had evidence of West Nile virus infection; 2 recipients were not available for testing.

The 16 implicated donors ranged from 18 to 72 years of age (median, 45.5); 10 were female (Table 3). Fourteen of the 16 implicated donors returned for a follow-up serologic analysis and in-

Table 3. Demographic, Clinical, and Laboratory Data for Donors Implicated in the Transfusion-Related Transmission of West Nile Virus in the United States, 2002.

Donor No.	Age (yr)/ Sex	Date of Donation	Time of Symptom Onset <i>days before or after donation</i>	Symptoms Reported*										Analysis of Sample Obtained at Donation†				
				Fever	Chills	Headache	Eye Pain	Muscle Pain	Swollen Glands	New Rash	New Thinking	Difficulty	Generalized Weakness	Muscle Weakness	Joint Pain	Abdominal Pain	Retention Segment	Plasma
1	44/F	July 22	7 to 14 Before‡	+	+	+	+	+	+	+	+	-	-	-	+	+	45.22	5.9§
2	27/F	Aug. 15	2 After	+	-	-	-	-	-	-	-	+	+	-	-	-	0.57	3.5
3	56/F	Aug. 30	—	-	-	-	-	-	-	-	-	-	-	-	-	-	Negative	10.5
4	65/M	Sept. 6	1 After	+	+	+	+	+	+	+	+	-	-	-	+	+	0.78	60.1
5	54/F	Aug. 23	5 Before to 4 after‡	+	+	+	+	+	+	+	+	-	-	-	+	+	11.21	NA
6	65/F	Aug. 21	1 to 21 After‡	+	+	+	+	+	+	+	+	-	-	-	+	+	Equivocal	NA
7	44/F	Sept. 9	11 After	+	+	+	+	+	+	+	+	-	-	-	+	+	Negative	0.8¶
8	46/F	Aug. 19	0	+	+	+	+	+	+	+	+	+	+	+	+	+	6.34	75.1§
9	47/M	Sept. 20	—	-	-	-	-	-	-	-	-	-	-	-	-	-	Equivocal	1.4
10	27/F	Aug. 31	5 After	+	-	+	-	+	-	+	-	-	-	-	+	+	Negative	39.8
11	32/M	Sept. 18	—	-	-	-	-	-	-	-	-	-	-	-	-	-	Equivocal	NA
12	18/M	Oct. 6	—	-	-	-	-	-	-	-	-	-	-	-	-	-	NA	4.6
13	25/M	July 25	14 to 15 Before‡	+	-	+	+	+	+	+	+	+	+	+	+	+	Negative	21.9
14	45/F	Sept. 25	—	-	-	-	-	-	-	-	-	-	-	-	-	-	Negative	0.8¶
15**	72/F	Aug. 19	—	-	-	-	-	-	-	-	-	-	-	-	-	-	Negative	11.9§
16**	46/M	Sept. 16	—	-	-	-	-	-	-	-	-	-	-	-	-	-	Negative	13.2§

* Plus signs indicate that the donor reported the symptom, and minus signs that the donor did not report the symptom.
 † "Retention segment" refers to the tubing attached to the original collection bag or the red-cell component. NA denotes sample not available for testing.
 ‡ The donor could not recall the exact time of onset of symptoms, so the date is given as a range.
 § West Nile virus was isolated from the unit of plasma.
 ¶ The polymerase-chain-reaction (PCR) assay was positive only when a more sensitive assay was used.
 || The leukoreduced red-cell unit from this donation tested negative on PCR.
 ** Virus was isolated from an initial donation sample; the donor did not return for a follow-up serologic analysis or interview.

terview. Nine of the 14 donors (64 percent) recalled having symptoms compatible with the presence of a viral illness: 3 began to have symptoms before donation, 1 began to have symptoms on the day of donation, and 5 first had symptoms after donation (Table 3). Three of the six donors who became ill on the day of or day after donation sought care from a physician. Symptomatic and asymptomatic donors were similar in age (median, 44 and 45 years, respectively). The symptoms of the implicated donors were compared with those of 654 nonimplicated donors who were IgM-negative at follow-up (Table 4). Multivariate analysis indicated that fever (adjusted odds ratio, 31.0; 95 percent confidence interval, 8.8 to 109.5), new rash (adjusted odds ratio, 11.0; 95 percent confidence interval, 1.9 to 65.3), and painful eyes (adjusted odds ratio, 4.6; 95 percent confidence interval, 1.04 to 20.5) were independently associated with being an implicated donor with West Nile virus infection.

Samples obtained at the time of donation from the 16 implicated donors had virus levels of less than 80 pfu per milliliter (Table 3); all were negative for West Nile virus IgM antibody. The estimated mean virus levels in retrieved plasma units from seven symptomatic donors and four asymptomatic donors (29.6 and 4.3 pfu per milliliter, respectively; $P=0.06$ by t-test) did not differ significantly. In individual donors, virus levels in retention segments differed from those in plasma (Table 3). Blood in retention segments was often of poor quality owing to hemolysis, dilution with red-cell preservative and saline, storage at room temperature, and filtration and leukoreduction procedures. When more than one sample from an initial donation was available for testing, all retention segments that were positive for West Nile virus on PCR were associated with a PCR-positive plasma sample, but in five instances, the plasma tested positive and the retention segment tested negative.

DISCUSSION

Our investigations document the transmission of West Nile virus through the transfusion of platelets, leukoreduced and nonleukoreduced red cells, and fresh-frozen plasma. Samples obtained from implicated donors at the time of donation had low levels of West Nile virus, which were sometimes near the limits of sensitivity of current nucleic acid-amplification assays, and none had IgM antibodies. Despite these low levels of virus, once transmis-

Table 4. Symptoms Compatible with West Nile Virus Infection Reported by Implicated Donors and IgM-Negative Nonimplicated Donors.*

Symptom	Implicated Donor	Nonimplicated Donor	P Value	Crude Odds Ratio (95% CI)
	(N=14) †	(N=654)		
	<i>no. (%)</i>			
Abdominal pain	2 (14)	15 (2.3)	0.05	7.1 (1.5–34.5)
Bone pain	0	12 (1.8)	—	—
Chills	6 (43)	22 (3.4)	<0.001	21.5 (6.9–67.4)
Fever	9 (64)	24 (3.7)	<0.001	47.3 (14.7–151.7)
Generalized weakness	8 (57)	32 (4.9)	<0.001	25.9 (8.5–79.2)
Headache	8 (57)	114 (17.4)	0.001	6.3 (2.2–18.6)
Joint pain	4 (29)	48 (7.3)	0.02	5.1 (1.5–16.7)
Muscle weakness	3 (21)	23 (3.5)	0.01	7.5 (2.0–28.7)
New rash	4 (29)	12 (1.8)	<0.001	21.4 (5.9–77.9)
New difficulty thinking	2 (14)	9 (1.4)	0.02	11.9 (2.3–61.3)
Painful eyes	5 (36)	31 (4.7)	<0.001	11.2 (3.5–35.3)
Seizures	0	1 (0.2)	—	—
Severe muscle pain	2 (14)	23 (3.5)	0.09	4.6 (0.97–21.6)
Swollen glands	3 (21)	14 (2.1)	0.004	12.5 (3.1–49.7)
Vomiting and diarrhea	0	27 (4.1)	—	—

* P values were calculated with use of Fisher's exact test. CI denotes confidence interval.

† Two implicated donors did not return for follow-up serologic analyses or interview.

sion was documented in one recipient, follow-up testing of all recipients of co-components demonstrated IgM antibody, indicating that transmission was highly efficient.

Many transfusion recipients were immunocompromised owing to treatment with immunosuppressive drugs or the presence of a hematologic or other advanced cancer. Little is known about the clinical outcome of West Nile virus infection in immunocompromised patients. Nearly 50 years ago, experimental West Nile virus infection of patients with cancer showed that patients with hematologic cancer had prolonged viremia.¹⁵ More recently, study of a patient who had non-Hodgkin's B-cell lymphoma and West Nile virus infection and of four recipients of organs from a West Nile virus-infected donor indicated that immunocompromised patients may have long incubation periods, prolonged viremia, delayed development of antibody, and an increased likelihood of severe disease.^{11,16} Incubation periods among recipients who were receiving immunosuppressive medication after organ, stem-cell, or bone marrow transplantation tended to be longer than those among recipi-

ents without immunocompromising conditions and longer than the generally accepted incubation period of 2 to 14 days among otherwise healthy persons after mosquito-borne transmission.^{5,6} Moreover, in specimens from seven immunocompromised recipients, we found West Nile virus RNA in serum or cerebrospinal fluid 7 to 31 days after the transfusion of the implicated component. West Nile virus nucleic acid–amplification tests in serum generally have very low sensitivity among otherwise healthy patients with meningoencephalitis.⁵

The 23 confirmed cases we describe most likely underrepresent the number of transfusion-transmitted West Nile virus infections that occurred during 2002 in the United States.⁴ Cases could have gone unrecognized because recipients remained asymptomatic, had West Nile virus–related illnesses that were indistinguishable from their underlying illnesses, or died from the underlying illness before West Nile virus–related illness developed. Physicians may not have considered blood transfusion as a source of infection, particularly in areas in which mosquito-borne transmission is prevalent. Some cases that we classified as inconclusive may have been transfusion-transmitted but could not be confirmed owing to the lack of complete information.

Although approximately 80 percent of West Nile virus infections are asymptomatic,¹⁷⁻¹⁹ during a follow-up interview, 9 of the 16 implicated donors recalled having a symptomatic illness around the time of donation. Although potential blood donors are not typically questioned about specific symptoms, they are asked whether they “feel well and healthy” and they do have their temperature measured. All of the implicated donors had successfully completed the donor-screening process on the day of donation. Symptoms reported by implicated donors were most likely related to West Nile virus infection, since they were reported much more frequently by implicated than by nonimplicated donors. Our results should have been unbiased, since West Nile virus testing was performed after the donors were interviewed. However, the exact timing of the onset of symptoms should be interpreted with caution, since the donors were questioned weeks or months after donation. One hypothesis to explain the high proportion of symptomatic donors is that symptomatic persons may have a greater level or longer duration of viremia than asymptomatic persons, thus making transfusion-related transmission more likely. In our investigations, levels of viremia tended to

be higher in symptomatic than in asymptomatic implicated donors, but this difference did not achieve statistical significance.

Our findings suggest that it may be useful to question potential donors about a recent history of febrile illness and ask those with such a history to defer donation. To help identify donors potentially at risk for West Nile virus infection, the Food and Drug Administration issued recommendations to blood-collection agencies in May 2003, which included the use of a new question to donors about a history of fever with headache in the week before donation; an affirmative answer would trigger deferral.²⁰

Our findings also suggest a potential benefit of West Nile virus nucleic acid–based screening of blood donors. Documentation of transfusion-transmitted West Nile virus infection stimulated the rapid development of an investigational nucleic acid–based assay suitable for donor screening. These assays began to be implemented in June 2003.²¹ As of July 14, 2003, all civilian blood donations collected in the United States and Puerto Rico have been screened for West Nile virus with the use of investigational nucleic acid–amplification tests. Of the approximately 1 million donations screened as of August 5, 2003, a total of 163 donations were found to be repeatedly reactive for West Nile virus and were removed from the blood supply.²²

Because of the low-level viremia associated with West Nile virus infection in humans, the sensitivity of donor screening assays will require careful assessment, including continued surveillance for possible transfusion-associated cases. Prompt reporting of such cases will facilitate withdrawal of potentially infectious blood components. The long-term benefit of screening of blood donors for West Nile virus is unknown. The risk of transfusion-transmitted West Nile virus infection relates to the incidence of the infection in the donor pool⁴; however, there are currently insufficient data to predict the incidence among future donors.

Although the blood supply in the United States has attained an unprecedented level of safety, it remains vulnerable to emerging infectious agents.²³ Documentation of the first 23 identified cases of transfusion-transmitted West Nile virus is a cogent reminder of that risk and highlights the responsibilities shared by local, state, and federal health agencies, blood-collection and transfusion establishments, health care providers, and industry to be vigilant for and respond to new pathogens.

We are indebted to B. Acquillino, A. Adams, C. Adamson, S. Allmond, D. Almonte, E. Alvarez, A. Banks, V. Barner, R. Berry, B. Biggerstaff, T. Boyd, L. Brooke, R. Brown, D. Brownell, L. Bumgarner, J. Carroll, M. Casey, B. Cermak, A. Chunn, K. Clark, A. Comer, J. Conway, R. Coombs, J. Duffy, B. Evans, G. Fink, M. Fornadley, S. Gates, L. Gifford, L. Gladden, S. Gordon, S. Gordon, G. Griffin, J. Haehn, S. Hand, J. Hanrahan, C.I. Hardesty, S. Harper, G. Hoeltge, C. Hooper, G. Howard, J. Howell, K. Howell, C. Huang, W. Icenhower, D. Jernigan, P. Jett, B. Johnston, J. Jones, A. Jordan, M. Keene, K. Klega, M. Kornman, P. Korth, L. Kramer, A. LaMonte, M. Larocco, B. Lattimore, P. Le, R. Lewis, J. Linden, S. Lowther, N. Luginbill, J. Marx, J. McCallum, D. McElroy, D. McKinney, K. McMilin, L. Mehl, A. Mil-

ler, W. Miller, C. Mize, A. Mizzi, L. Montague, T. Morris, R. Moseley, J. Moy, B. Mudd, M. Naber, D. Newman, M. O'Connor, R. Oesterle, N. Pascoe, C. Perego, D. Phillips, T. Pishivili, S. Porter, J. Ramsey, P. Rawlins, K. Reid, C. Revere, J. Robb, J. Robertson, J. Roche, D. Rosecrans, S. Rossmann, L. Russell, Z. Sahl, R. Sassetti, B. Schable, J. Schuermann, L. Scott, R. Shallenberger, B. Shipley, F. Shoaf, K. Singletary, K. Smith, S. Snow, S. Spieldenner, M. Spradlun, J. Squires, L. Stark, W. Stephenson, B. Thompson, G. Thompson, D. Tillman, S. Trice, J. Trotter, D. Vina, J. Waggoner, A. Wagner, L. Wagner, N. Washington, J. Watson, D. Webb, M. Weber, D. Wilkinson, Y. Williams, P. Williamson, P. Willson, M. Wilson, E. Woodland, S. Wong, A. Zak, and K. Zimmerman.

APPENDIX

The following were members of the West Nile Virus Transfusion Transmission Investigation Team: Agency for Toxic Substances and Disease Registry, Atlanta — C. Noonan; American Red Cross — G. Baker, J. Burch, D. Carroll, T. Carruthers, C. Coddington, S. Ellison, M.C. Fucci, J. Gipple, P. Griffith, J. Griggs, P. Hartnett, K. Hillyer, L. James, C. Meena-Leist, L. Mott, B. Noeyack, K. Peterson, R. Reddy, P. Royster, S. Sapatnekar, K. Skidmore, C. Thomas, M.E. Wissel; Alabama Department of Public Health, Montgomery — A. Becker; Alachua County Health Department, Gainesville, Fla. — S. Bethart, T. Belcuore, J. Shapiro; Blood Systems, Scottsdale, Ariz. — H. Kamel; Bureau of Laboratories, Michigan Department of Community Health, Lansing — P. Clark, F. Downes, J. Massey, P. Somsel; Centers for Disease Control and Prevention — K. Abe, W. Bower, T. Chambers, L.E. Chapman, P. Collins, V. Elko, A. Grant, T. Harrington, G. Huhn, T.B. Hyde, M.C. Iwamoto, A.J. Johnson, J.L. Jones, O.I. Kosoy, M.J. Kuehnert, A. MacNeil, D.A. Martin, P. Mead, J. Montgomery, S. Neff, A.J. Noga, J.T. Roehrig, C. Van Beneden, A. Vicari, A. Winquist, D. Withum; Food and Drug Administration, Rockville, Md. — E. Callaghan, J. Davis, J. Epstein; Florida Department of Health, Tallahassee — L. Conti; Heartland Blood Centers, Aurora, Ill. — D. Bazile, K. Clark, K. Konrad, D. Mestrach, S. Mitzelfelt; Life Source Blood Services, Glenview, Ill. — R. Kakaiya, R. Tata; LifeSouth Community Blood Centers, Gainesville, Fla. — J. Evans; Maryland Department of Health and Mental Hygiene, Baltimore — A. Bergmann, D. Blythe, R. Myers; Mecklenburg County Health Department, Charlotte, N.C. — S. Keener; MeritCare Health System, Bismarck, N.D. — J. Cook; Mississippi State Department of Health, Jackson — M. Currier; New York State Department of Health, Albany — M. Anand; North Dakota Department of Health, Bismarck — K. Kruger, T. Miller, L. Shireley; Ohio Department of Health, Columbus — K. Wimpisinger; Oklahoma Blood Institute, Oklahoma City — J. Smith; Oklahoma State Department of Health, Oklahoma City — K. Bradley; University of Michigan Medical Center, Ann Arbor — R. Davenport, D. Newton; Wisconsin Division of Public Health, Madison — L. Glaser.

REFERENCES

- Nash D, Mostashari F, Fine A, et al. The outbreak of West Nile virus infection in the New York City area in 1999. *N Engl J Med* 2001;344:1807-14.
- Roehrig JT, Layton M, Smith P, Campbell GL, Nasci R, Lanciotti R. The emergence of West Nile virus in North America: ecology, epidemiology, and surveillance. *Curr Top Microbiol Immunol* 2002;267:223-40.
- Provisional surveillance summary of the West Nile virus epidemic — United States, January–November 2002. *MMWR Morb Mortal Wkly Rep* 2002;51:1129-33.
- Biggerstaff BJ, Petersen LR. Estimated risk of West Nile virus transmission through blood transfusion during an epidemic in Queens, New York City. *Transfusion* 2002;42:1019-26.
- Petersen LR, Marfin AA. West Nile virus: a primer for the clinician. *Ann Intern Med* 2002;137:173-9.
- Campbell GL, Marfin AA, Lanciotti RS, Gubler DJ. West Nile virus. *Lancet Infect Dis* 2002;2:519-29.
- Harrington T, Kuehnert MJ, Kamel H, et al. West Nile virus infection transmitted by blood transfusion. *Transfusion* 2003;43:1018-22.
- West Nile virus infection in organ donor and transplant recipients — Georgia and Florida, 2002. *MMWR Morb Mortal Wkly Rep* 2002;51:790.
- Update: investigations of West Nile virus infections in recipients of organ transplantation and blood transfusion. *MMWR Morb Mortal Wkly Rep* 2002;51:833-6.
- West Nile virus activity — United States, October 10–16, 2002, and update on West Nile virus infections in recipients of blood transfusions. *MMWR Morb Mortal Wkly Rep* 2002;51:929-31.
- Iwamoto M, Jernigan DB, Guasch A, et al. Transmission of West Nile virus from an organ donor to four transplant recipients. *N Engl J Med* 2003;348:2196-203.
- Epidemic/epizootic West Nile virus in the United States: revised guidelines for surveillance, prevention, and control. Atlanta: Centers for Disease Control and Prevention, 3rd revision, 2003. (Accessed September 2, 2003, at <http://www.cdc.gov/ncidod/dvbid/westnile/resources/wnv-guidelines-aug-2003.pdf>.)
- Lanciotti RS, Kerst AJ, Nasci RS, et al. Rapid detection of West Nile virus from human clinical specimens, field-collected mosquitoes, and avian samples by a TaqMan reverse transcriptase-PCR assay. *J Clin Microbiol* 2000;38:4066-71.
- Martin DA, Muth DA, Brown T, Johnson AJ, Karabatsos N, Roehrig JT. Standardization of immunoglobulin M capture enzyme-linked immunosorbent assays for routine diagnosis of arboviral infections. *J Clin Microbiol* 2000;38:1823-6.
- Southam CM, Moore AE. Induced virus infections in man by the Egypt isolates of West Nile virus. *Am J Trop Med Hyg* 1954;3:19-50.
- Huang C, Slater B, Rudd R, et al. First isolation of West Nile virus from a patient with encephalitis in the United States. *Emerg Infect Dis* 2002;8:1367-71.
- Tsai TF, Popovici F, Cernescu C, Campbell GL, Nedelcu NI. West Nile encephalitis epidemic in southeastern Romania. *Lancet* 1998;352:767-71.
- Mostashari F, Bunning M, Kitsutani PT, et al. Epidemic West Nile encephalitis, New York, 1999: results of a household-based seroepidemiological survey. *Lancet* 2001;358:261-4.
- Serosurveys for West Nile virus infection — New York and Connecticut counties, 2000. *MMWR Morb Mortal Wkly Rep* 2001;50:37-9. [Erratum, *MMWR Morb Mortal Wkly Rep* 2000;50:101.]
- Guidance for industry: revised recommendations for the assessment of donor suitability and blood and blood product safety in cases of known or suspected West Nile virus infection. Rockville, Md.: Food and Drug Administration, May 2003. (Accessed September 2, 2003, at <http://www.fda.gov/cber/gdlns/wnvguid.htm>.)
- Blood centers begin implementing WNV donor screening tests. ABC Newsletter. June 20, 2003;1-3. (Washington, D.C.: America's Blood Centers.)
- Detection of West Nile virus in blood donations — United States, 2003. *MMWR Morb Mortal Wkly Rep* 2003;52:769-71.
- Chamberland ME. Emerging infectious agents: do they pose a risk to the safety of transfused blood and blood products? *Clin Infect Dis* 2002;34:797-805.

Copyright © 2003 Massachusetts Medical Society.