Detection of West Nile virus in the Mexican blood supply

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BACKGROUND: West Nile virus (WNV) is the etiologic agent of an emerging disease in the Western Hemisphere that can be transmitted to humans by blood transfusion. WNV first appeared in the United States in 1999, in Canada in 2001, and in Mexico in 2002. The aim of this nationwide study was to determine the prevalence of WNV in blood donors in Mexico as a first step in preventing its transfusion-associated transmission.

STUDY DESIGN AND METHODS: In July and August 2004, a total of 3856 fresh plasma specimens collected from each state's center for blood transfusion in 29 of 31 Mexican states were screened with an investigational WNV assay (Procleix,® Gen-Probe Inc. and Chiron Corp.), a nucleic acid test based on transcription-mediated amplification (TMA). Reactive specimens were confirmed with a second TMA-based test, the alternative WNV assay (Gen-Probe), and with WNV capture enzyme-linked immunosorbent assays (ELISAs) for detection of immunoglobulin M (IgM) and IgG antibodies. In addition, 3714 frozen plasma samples collected in 2002 and 2003 were similarly tested.

RESULTS: One of 3856 fresh samples from an asymptomatic donor from Chihuahua was reactive by both TMA-based tests and IgM ELISA, suggesting a recently acquired infection. The observed percentage of viremic donors blood donors was 0.03 percent. Results from frozen samples were not included in the prevalence calculation and none were TMA-reactive for WNV.

CONCLUSIONS: WNV is present in the Mexican blood supply and measures should be taken to reduce the risk of transfusion transmission.

Protection of a country's blood supply is a dynamic process of critical importance. Health officials must be continually aware of new emerging pathogens, not only because of their potential direct impact on the population, but also because of their potential to contaminate the blood supply and cause transfusion-associated illnesses. With today's increased travel, pathogens from remote corners of the world can suddenly emerge in another continent, quickly taking hold and sometimes infecting the blood supply. In 2002, the Mexican health authorities became aware of such a threat from the West Nile virus (WNV), which was rapidly spreading across the continental United States and Canada.[1-6]

In 1999 WNV, originally identified in Uganda in 1937, made its first appearance in North America in New York where it was associated with an encephalitis outbreak.[5] The viral strain responsible for the epidemic was a lineage 1 virus that had appeared in Israel between 1997 and 2000.[6-9] The WNV is single-stranded RNA virus that belongs to the Japanese encephalitis serocomplex of the Flaviviridae family. It is maintained in an enzootic cycle that involves culcine mosquitoes and birds. The most common route of infection for humans is through the bite of an infected *Culex* mosquito.[3,6] Vertical transmission and other transmission routes, however, including via breast-feeding, organ transplantation, and transfusion have also

ABBREVIATIONS: s/co ratio = signal-to-cutoff ratio; TMA = transcription-mediated amplification; WNV = West Nile virus.

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been documented.[1,3,6] Humans and other mammals, such as horses, are incidental hosts, because they only develop transient low levels of the virus, which are usually insufficient to infect mosquitoes. Despite this low-grade viremia, approximately 20 percent of infected humans develop West Nile fever, a febrile illness of sudden onset, often associated with a lengthy recovery period. Approximately 1 in 150 develop neuroinvasive disease in the form of encephalitis and/or meningitis. The reported case fatality rate for the infection varies from 4 to 14 percent with those over age 50 at highest risk.[7]

After its appearance in New York, the WNV began to spread across the continental United States, becoming the largest arbovirus epidemic in recorded history.[10] In 2000, the Centers for Disease Control and Prevention in cooperation with state and local health departments established an electronic surveillance system, ArboNet, for tracking WNV infections in mosquitoes, birds, humans, and other mammals in the United States 1 year before the virus appeared in the southern Canadian province of Ontario and started spreading through Canada. As of October 2004 it was estimated that approximately 940,000 people in the United States had been infected with WNV and that 190,000 had become ill.[1]

In response to the rapid spread of the virus across the North American continent and its establishment in Texas by 2002, Mexican health authorities anticipated the emergence of the virus in Mexico. Indeed reports were emerging of encephalitis outbreaks in horses in Mexican states that bordered Texas. Because equine and avian infection have often preceded human infection, the authorities conducted an equine and avian infection surveillance study in 14 Mexican states during the summer of 2002. WNV-specific antibodies were found in 22 percent of equine serum samples from herds with a history of encephalitis in six Mexican states, three of which border Texas. In addition, WNV was isolated from a dead common raven in one of the six states.[11-13] Phylogenetic studies suggested that the strain of WNV found likely came from the central United States.[11,14] Mexico was now on the alert for the emergence of WNV in the human population.

In the meantime, as the virus was now documented in the US population, an American investigational team undertook a lookback study the following year that documented 23 cases of transfusion-associated transmission of WNV. These 23 cases were linked to 16 viremic donors, all of whom were negative for WNV-specific immunoglobulin M (IgM) antibodies at the time of their donation.[15] Serology-based assays that had been previously used for diagnosis of WNV infection were now not considered to be appropriate for blood screening, because they detect the host immune response that usually appears after the brief 6- to 11-day viremic phase.[16] By the summer of 2003, the US government and industry had cooperated to develop and implement nationwide investigational nucleic acid testing (NAT) of blood donors for WNV.[17] Subsequently, the Food and Drug Administration (FDA) developed a questionnaire designed to detect potentially infected donors and refined an algorithm for switching from testing minipools to individual donors.[18] Although guidance documents for WNV confirmatory testing are still under development, confirmation of a viremic donor currently involves testing with a secondary NAT followed by specific antibody tests and, in some instances, specific plaque reduction neutralization tests (J. Bishop, Center for Biologics Evaluation and Research, US FDA, personal communication).

In light of the rapid spread of WNV in the United States and Canada and the documented threat it continues to pose to those countries' blood supplies, we decided to determine if the virus had entered our human population and, in particular, our blood supply. We performed a nationwide study of blood donor samples to estimate the prevalence of WNV infection among the different Mexican states and to investigate mechanisms for prevention of its transfusion-associated transmission. We used an investigational NAT, the Procleix® WNV assay (Chiron Corp., Emeryville, CA) that is based on transcription-mediated amplification (TMA), to screen for WNV RNA in 3856 fresh plasma specimens from 29 of 31 Mexican states and in 3714 frozen repository plasma specimens donated to numerous blood collection agencies in 2002 and 2003. Initially reactive samples were retested with an alternative WNV confirmatory assay and with WNV enzyme-linked immunosorbent assays (ELISAs) for IgM and IgG antibodies.

MATERIALS AND METHODS

Collection of fresh samples and study design

Between July 17 and August 27, 2004, we collected 4234 fresh plasma samples from consecutive healthy blood donors who presented at their State Center for Blood Transfusion in 29 of 31 Mexican states for the purpose of determining the prevalence of WNV in the Mexican blood donor population. All samples collected between those dates were included in the study except for those with evidence of inadequate centrifugation, hemolysis, fatty supernatant, inappropriate handling, or insufficient volume. Three-hundred seventy-eight (9%) were excluded. A total of 3856 fresh samples met study inclusion criteria. Each state contributed between 98 and 157 samples, with a mean donation of 136 samples. Two states, Baja California Sur and Tabasco, were unable to participate in the study, because reorganization in their health systems coincided with the study start. Ninety-nine percent of the participants were first-time donors. All donors participating in the study gave written informed consent. Each had a complete medical history taken by the blood bank physician in accordance with Mexican health regulations and

each completed a questionnaire designed to detect symptomatic donors with WNV.[19,20] The study was approved by the ethical and research committee at the National Center for Blood Transfusion. Samples were collected in PPT tubes (Vacutainer, Becton Dickson, Franklin Lakes, NJ) and the plasma was stored at 2 to 8°C between 24 and 124 hours until tested. Specimens were handled as directed in the Procleix WNV assay package insert. Testing was performed in minipools composed of 16 individual samples. Each member of an initially reactive pool was retested individually to identify the reactive donation.

Collection and screening of previously frozen samples

In addition, previously frozen specimens stored at –30°C were also screened as individual samples. These were collected from consecutive donors at the National Center for Blood Transfusion in Mexico City from February 2002 to April 2003 during the performance of a prospective, nonrandomized, unblinded study to determine the seroprevalence of hepatitis C virus (HCV).[21] A total of 2887 samples were collected in 2002 and 827 in 2003. Samples were excluded only for the same conditions as noted above for the fresh samples. From a total of 4157 frozen samples, 3714 were included in the study. Four-hundred forty-three frozen samples were excluded by the same criteria as used for the fresh samples. Only 10 (0.3%) samples in this study group were obtained from repeat donors.

WNV RNA screening methods

A total of 3856 fresh and 3714 frozen samples were tested by the Procleix® WNV assay, a qualitative TMA-based NAT designed to detect WNV RNA. This assay has been previously described and is currently being utilized to screen blood donations in the United States under the FDA investigational new drug mechanism.[22,25] Samples with signal-to-cutoff (s/co) ratios of ≥1.0 were considered reactive and underwent further nucleic acid and antibody confirmatory testing.[26] Fresh samples were tested in minipools composed of 16 individual samples. A total of 241 minipools were tested. In the event of an initially reactive result, separate aliquots from each of the 16 pool members were retested with the Procleix WNV assay to determine the identity of the potentially viremic donor. Repeat-reactive samples were subsequently individually tested by the alternative WNV assay, a confirmatory investigational NAT developed by Gen-Probe, also based on TMA technology, but with primers and probes that target a different region of the WNV genome than those of the Procleix WNV assay.[27] The performance of both of these NATs has been previously described.[28] All frozen samples were initially tested individually and the reactive ones were further tested, employing the same algorithm as used for the fresh samples.

IgG and IgM antibody testing

All fresh and frozen samples that were reactive by the Procleix WNV assay were also tested for both IgG and IgM antibodies to WNV with the IgM capture ELISA and the IgG direct ELISA (Focus Technologies, Cypress, CA).[29,30]

Follow-up of NAT-reactive donors

We obtained the medical history of reactive donors and recipients of any of their blood products. Health authorities in each state were notified of confirmed viremic donors. They surveyed those living in the vicinity of viremic donors and drew blood samples from their close contacts. Although the authorities did not test animals, birds, or mosquitoes in the area, they subsequently administered a questionnaire designed to detect symptomatic infections to 3900 people living within a 49-block perimeter of the viremic donor's home. Additionally, blood samples were obtained from two of the viremic donor's close contacts.

RESULTS

Fresh samples

Of the 241 minipools tested, 3 were initially reactive by the Procleix WNV assay. On individual testing of each of the 48 members of the 3 initially reactive pools, only 1 was repeatedly reactive by the Procleix WNV assay. This member subsequently was confirmed positive by the alternative WNV test and also had detectable IgM antibodies to WNV, although it had no detectable IgG antibodies. Unfortunately, there was insufficient sample volume for sequencing. Sample details are shown in Table 1.

Determination of observed percentage of viremic blood donors

Only donors confirmed viremic by the alternative WNV test from the population of fresh specimens were included in the observed percentage calculation. The observed percentage among this population was calculated as 1 per 3856 or 0.03 percent. The one confirmed viremic donor came from the northern state of Chihuahua, which contributed 4.3 percent of all Mexican blood donations in 2004 and contributed a total of 146 samples to this study. The observed percentage of WNV-infected blood donors for the state of Chihuahua was calculated as 1 per 146 or 0.7 percent.

TABLE 1. WNV screening and confirmatory test results

Sample identification	Donation date	Donor residence (state or city)	Procleix WNV s/co ratio	Procleix WNV Result	Alternative WNV s/co ratio	Alternative WNV Result	ELISA IgM	ELISA IgG
Fresh samples								
13421	August 1, 2004	Chihuahua	26.22	Reactive	11.0	Reactive	Reactive	Nonreactive
16537	August 10, 2004	Baja California	25.61	Reactive	0.03	Nonreactive	Nonreactive	Nonreactive
18071	August 17, 2004	Guerrero	1.46	Reactive	0.04	Nonreactive	Nonreactive	Nonreactive
Frozen samples								
3593	March 24, 2002	Mexico	1.13	Reactive	0.03	Nonreactive	Nonreactive	Nonreactive
4366	April 13, 2002	Mexico City	22.19	Reactive	0.03	Nonreactive	Nonreactive	Reactive
5463	May 1, 2002	Colima	1.63	Reactive	0.07	Nonreactive	Nonreactive	Nonreactive
9326	July 21, 2002	Mexico	1.63	Reactive	0.07	Nonreactive	Nonreactive	Nonreactive
9967	August 3, 2002	Mexico City	1.97	Reactive	0.05	Nonreactive	Nonreactive	Nonreactive
14736	November 9, 2002	Mexico	2.21	Reactive	0.03	Nonreactive	Nonreactive	Nonreactive
15030	November 17, 2002	Mexico City	2.26	Reactive	0.03	Nonreactive	Nonreactive	Nonreactive
15122	November 20, 2002	Mexico City	1.00	Reactive	0.03	Nonreactive	Nonreactive	Nonreactive
15145	November 20, 2002	Mexico	1.05	Reactive	0.04	Nonreactive	Nonreactive	Nonreactive
15536	November 30, 2002	Mexico	1.05	Reactive	0.06	Nonreactive	Nonreactive	Nonreactive
15586	December 1, 2002	Mexico City	1.00	Reactive	0.04	Nonreactive	Nonreactive	Nonreactive

Frozen samples

Eleven of 3714 frozen samples were initially reactive by the WNV Procleix assay. Of these 11, none could be confirmed by the alternative WNV assay. One was IgG-reactive, but unfortunately the donor refused to given an additional sample for plaque reduction neutralization testing considered necessary for confirmation of WNV exposure. Sample details are shown in Table 1.

Follow-up histories on reactive donors

The one confirmed viremic sample was obtained from a 41-year-old male farmer from Aldama, a central county in the state of Chihuahua. This finding is not surprising given that his home is close to a river that courses through a bird sanctuary that has a dense population of mosquitoes. He was asymptomatic at the time of interview and had not been ill in the past year or traveled in the past 16 years. Because his routine serologic markers for human immunodeficiency virus (HIV), hepatitis B virus, and HCV were negative, his blood was processed into components and released. A 2-year-old child with Kwashiorkor received a unit of his fresh-frozen plasma and died 3 days after the transfusion of her malnutrition. Although she had no symptoms of WNV infection at the time of her death, no blood samples were drawn for WNV testing. The donor's other blood products were subsequently discarded. At the time of this writing, the donor continues to be asymptomatic. A questionnaire administered by the personnel of the Center for Prevention and Disease Control in the State of Chihuahua to 3900 people living within a 49-block perimeter of the viremic donor's home did not suggest any additional human cases. Nevertheless, health promotion and environmental control programs were introduced in the community. Blood samples from two of the viremic donor's close contacts had insufficient volume for WNV testing and additional samples could not be obtained.

The location of the confirmed viremic donor is illustrated in Fig. 1, a map of the Mexican Republic and the American border states of California, Arizona, New Mexico, and Texas. The number of confirmed WNV viremic blood donations in the year 2004 for each of the American border states is indicated within each state boundary. The two nonparticipating Mexican states are also shown.

DISCUSSION

Emergent diseases such as Creutzfeldt-Jakob disease, severe acute respiratory syndrome, and WNV fever or encephalitis have received widespread attention in the past few years, resulting in significant advances in knowledge of their etiology, pathogenesis, and epidemiology, as well as their transfusion-associated transmission potential.[31-34] Recent reports have shown that WNV-infected donors, even those with very low levels of virus, can transmit the infection through blood or organ donations.[19,35-37] These reports have spurred intensified efforts to reduce the risk of WNV transfusion transmission through minipool donor screening and individual donor screening when possible.

NAT has already reduced the risk of transmission of HCV and HIV and prevented the transfusion of more than 1000 units infected with WNV in 2003.[18] In the United States and Canada, NAT donor screening for WNV has also proved useful for disease surveillance and prevention, because it helped track the epidemic in real time. It led to the prompt identification of infected donors, which in turn helped delineate the geographic

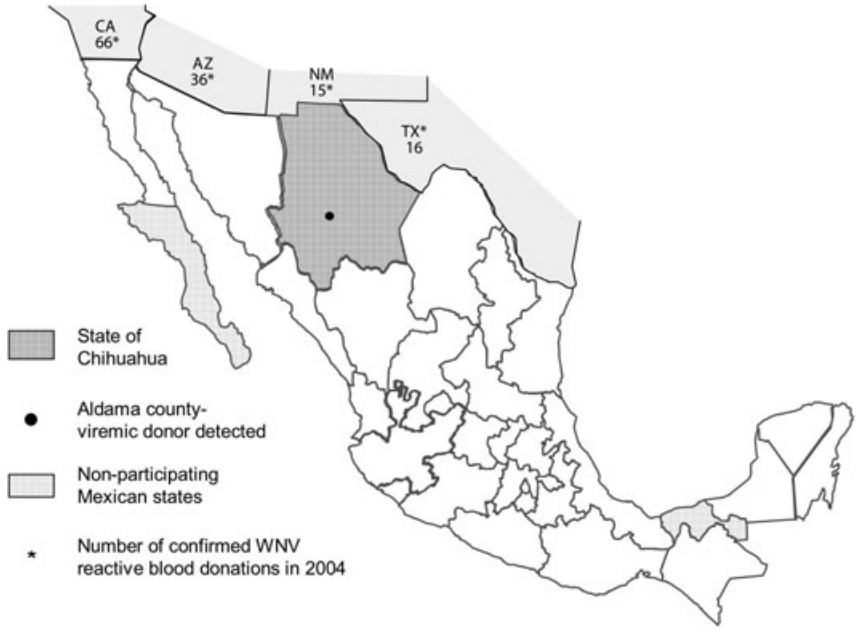

Fig. 1. Map showing Mexican states and American border states. (▭) State of Chihuahua and county of Aldama where confirmed viremic donor was identified (●) is indicated along with the two Mexican states that did not participate in the study (▭). *The number of confirmed WNV-reactive blood donations for each of the American states that border Mexico for the year 2004 are indicated within the boundary of the given state.

spread of the WNV often before clinical cases were reported.

Without this tool, identification of potentially infected blood donors in Mexico would have been most challenging. Even in countries like the United States where the population has not been exposed to other flaviviruses, the infection is largely asymptomatic. It is difficult to predict how WNV infection will manifest itself in our country where many people have had prior exposure to other flaviviruses. Such individuals could exhibit more severe disease because of immune enhancement, but more likely would exhibit attenuated WNV infection because of cross-protective antibodies.[11] Indeed before our study, WNV activity reported in Mexico was confined to birds and horses. Unpublished reports existed of eight suspected cases of human infection by WNV in the northern region of the country that had resulted in three cases of neuroinvasive disease and one death (P. Kuri, personal communication). There was no confirmed evidence of WNV in the human population, however, let alone in the blood supply.

Our sentinel study was the first performed in a population of healthy Mexican blood donors and the results are not surprising, given the spread of the virus through our northern neighbors. The presence of the virus both in the US human population and its blood supply has been well documented in 2003 and 2004. During the past year, 391, 88, 158, and 771 WNV cases and 36, 15, 16, and 66 infected blood donations were reported in the border states of Arizona, New Mexico, Texas, and California, respectively. The 1408 documented cases in these states represent 57 percent of the 2470 reported cases in the entire United States in 2004.[38] Our finding of one NAT-confirmed WNV-infected blood donor in the northern state of Chihuahua during the summer of 2004 indicates the virus is indeed present in the northern region of our country, which borders these American states. The fact that this donor was also WNV IgM-reactive and IgG-nonreactive suggests that he had a recent infection. Unfortunately, owing to insufficient sample volume, we were unable to perform genetic sequencing of the virus to determine its origin. Results from screening our previously frozen samples are less definitive because they represent a separate population collected at an earlier time, and for that reason we did not include them in our prevalence calculations. Owing to lack of sufficient sample volume for plaque reduction neutralization testing, we could not confirm the fact that the one sample that was reactive for WNV IgG antibodies represented a true past WNV infection where clearance of virus and IgM antibody had already occurred.

Although our study was based on a convenience sample, because only donations from each state's center for blood transfusion were tested as opposed to donations from every transfusion center, we believe in all likelihood that our sample is representative of the Mexican blood donor population. The 29 participating Mexican states contributed 97.3 percent of all blood donations collected in Mexico in 2004. The participating state centers, located in key cities, process blood collected both locally and from rural areas. Consecutive donors from each of these centers were tested over a 6-week time period and only those whose residual samples were degraded or had insufficient volume for further testing were excluded. In addition, all but 1 state contributed well over 100 samples each.

Nevertheless, we recognize that the extrapolation of our calculated observed percentage of viremic donors both in the state of Chihuahua and in the entire country of Mexico must be interpreted with caution, because it is based on screening only a mean of 136 donors from each state and a total of 3856 donors from the entire country. At the same time, our observed prevalences of WNV are similar to those reported in the United States in 2003. Our observed national percentage rate of 0.03 percent is close to the estimated US rate of

0.016 percent, based on 163 confirmed WNV-infected units of approximately 1 million units collected and screened between July 14 and August 5, 2003, in the continental United States and Puerto Rico.[15] Our observed percentage rate among donors from Chihuahua of 0.7 percent is in the midrange of the 0.1 to 1.1 percent prevalence rate based on screening more than 7000 specimens in North Dakota in the peak summer season of 2003, the initial year of that region's epidemic,[31] yet we have had no evidence of focal epidemics of WNV similar to those observed to our north. It is therefore possible that we have underestimated our observed blood donor percentage rate, because we cannot rule out the possibility that two of our initially reactive samples with s/co ratios above 22 that we were not able to confirm actually contained low levels of virus. Although there is not an absolute correlation between s/co ratios and the amount of virus in a sample, approximately half the samples with s/co ratios of greater than 20 are subsequently confirmed by alternative assays.[39]

Even without an exact prevalence determination, it appears that WNV is now present in Mexico. From the experience of other countries, it is probably here to stay. Because of our temperate, arid climate in the north, and our subtropical and tropical climates in the south, we might expect to observe year-round transmission of the virus in contrast to the limited summer season for transmission observed in colder climates.[40] In addition, the documentation of WNV antibody–positive animals in Puerto Rico, Jamaica, Dominican Republic, and Guadalupe suggests that WNV may be spreading to the south. Central and South American countries should be on the alert for its arrival in their areas over the next few years.[6]

Because WNV will probably become a permanent feature of the Mexican landscape, our public health system should be prepared to perform a larger surveillance of the population and have the requisite screening and confirmatory tests available. We should establish diagnostic criteria for WNV infection in our population and put in place a centralized reporting mechanism. By monitoring the activity of WNV infection in our country, we will learn when to initiate further studies and when, and if, we will need to put in place the protective measures to decrease the possible transfusion associated transmission of WNV.

Like other countries, we will strive to achieve an appropriate level of blood safety without consuming too great a share of our health service resources. We will need a clear decision-making pathway both to implement appropriate screening for WNV and to create the infrastructure for reporting presumed viremic donors to the health authorities.[41,42] In addition, we should refine our standard medical history taken of every potential blood donor to aid in the identification of symptoms that might be associated with WNV infection, so that some recently infected donors might be deferred.[18,41] Because most of infected donors are asymptomatic at the time of donation, we will eventually need a hemovigilance system that could trace released units from donors who become ill with WNV within a few weeks of donation or whose recipients become ill with presumed WNV infection.[15,41] Such a system would also include a donation call-back procedure and a mechanism for reporting adverse events. It could also help in preventing other emergent transfusion transmitted diseases we might encounter in the future. Because implementation of such a program will put an additional burden on our health-care system, we will need more information to decide when to undertake such screening and if it should be implemented year round or just on a seasonal basis. In the meantime, Mexican health authorities are urged to initiate an appropriate monitoring system, while assessing the feasibility of donor screening for WNV.

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