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West Nile Virus and Blood Product Safety in Germany

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West Nile Virus (WNV) is a mosquito-transmitted flavivirus, widely distributed throughout Africa, Asia and the Middle East. WNV may cause epidemics of human meningoencephalitis. The unexpected emergence of WNV (New York, 1999) and its rapid spread throughout North America during the following years caused a number of blood transfusion- and organ transplant-associated transmissions of WNV. In order to estimate the potential WNV threat for Central Europe, we analyzed the anti-WNV prevalence and WNV-RNA incidence among 14,437 and 9,976 blood donors from Germany. There was a high rate of initially anti-WNV reactives (5.9%), but only a few cases (0.03%) were confirmed as anti-WNV positive by neutralization assay. No WNV-RNA positive blood donor was identified in this study. Whereas WNV-RNA was frequently detected in manufacturing plasma pools from the US, none was detected in pools of European or Asian origin. Virus inactivation steps integrated into the manufacturing process of plasma derivatives were shown to be sufficient to assure the WNV safety of plasma derivatives. A well-characterized WNV reference material was prepared, showing 340 WNV-RNA copies per infectious dose. ***J. Med. Virol. 80:557–563, 2008.***

KEY WORDS: West Nile Virus; WNV neutralization; anti-WNV IgG ELISAs; blood safety; plasma derivatives; virus inactivation

INTRODUCTION

West Nile Virus (WNV) was identified in 1937 in Uganda and is widely distributed throughout Africa, Asia, the Middle East and parts of Europe [Solomon et al., 2003]. This enveloped virus is classified under the virus family *Flaviviridae* in the genus *Flavivirus*, which includes more than 100 members that all are characterised by a complex replication cycle involving both insects and at least one additional animal species. This is why flaviviruses had been included in the previous taxonomic group of arboviruses (arthropod-borne viruses). The natural WNV replication cycle involves *culicine* mosquitoes and different bird species. Humans, horses and other mammalian species are so-called "dead-end" hosts characterised by WNV infections with potential clinical symptoms, but transient and low virus levels that are insufficient to establish a mosquito-mammalian WNV replication cycle. The vast majority of WNV infections in humans undergo an asymptomatic course. Approximately 20% of infected humans develop West Nile fever, a febrile illness of sudden onset, often associated with a long recovery period. Only a few cases (<0.2%) develop a neuroinvasive disease resulting in more serious symptoms, including meningitis or encephalitis, sometimes with fatal outcome [Petersen and Marfin, 2002]. The rate of serious outcome of WNV infection is much higher in immune-compromised patients, a status more frequently found in elderly persons or in recipients of blood transfusions.

After a flavivirus infection, the diagnostic differentiation of specific antibodies is complicated by a high rate of cross-reactivity between different members of the genus Flavivirus, for example, Dengue virus (DenV), tick-borne encephalitis virus (TBEV), and WNV [Allwinn et al., 2002; Koraka et al., 2002]. Cross-reactive antibodies are mainly directed against an envelope protein of flaviviruses, the E-protein.

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The unexpected emergence of WNV in New York in 1999 was associated with an encephalitis outbreak. The viral strain responsible for this epidemic was a lineage 1 virus already known from previous epidemics in other parts of the world, such as in Israel [Lanciotti et al., 1999]. In the following years, WNV spread across North America from East to West, causing the largest arbovirus epidemic in recorded history with more than 23,000 human infection cases until December 2006, including 893 deaths [Centers for Disease Control and Prevention, 2006]. During this WNV epidemic, new transmission routes, including breast-feeding [MMWR, 2002], organ transplantation [Iwamoto et al., 2003] and transfusion of blood components [Biggerstaff and Petersen, 2002; Hollinger and Kleinman, 2003], were recorded. To assure the safety of the US blood supply, the screening of all blood donations by nucleic acid amplification techniques (NATs) was recommended by the FDA beginning in July 2003. This measure resulted in the detection of WNV-RNA in more than 1,000 blood donations until the middle of 2005, which would otherwise have been used for transfusion of non-inactivated cellular blood components (red cells, platelets) or therapeutic plasma [Busch et al., 2005a; Stramer et al., 2005].

Despite some reports about sporadic WNV infections in humans and horses across Europe, for example, in France, Italy, and Romania [Zeller et al., 2004], no WNV epidemiology data were available for Germany. With the US epidemic still ongoing, the question arose as to whether a similar scenario could also affect Europe. The German Ministry of Health initiated an investigation into the prevalence and incidence of WNV infections in Germany. This publication relates to the assessment of WNV safety of blood and plasma products used in Germany.

MATERIALS AND METHODS

Human Serum and Plasma Samples

For the evaluation of WNV prevalence, 14,437 plasma or serum samples were collected during Summer 2004 from healthy blood donors from central Germany (Hesse) and 928 samples from Austrian blood donors (Carinthia and Vienna).

For the determination of WNV incidence, plasma samples from 9,978 healthy blood donors from central Germany (Hesse) were collected during the 2005 summer mosquito season, combined in pools of 8 using a Tecan robot, and tested for the presence of WNV-RNA.

Serum samples from German intravenous drug users (IVDUs) who tested positive for other blood-borne viruses (HIV and/or HCV) were collected during May 2002 and January 2004 at University Hospital Frankfurt/Main.

Anti-WNV IgG-positive plasma samples were obtained from confirmed clinical cases in the US. These plasma samples were obtained from BBI (West Bridgewater, MA) or kindly provided by H. Hofmann (Genzyme Virotech GmbH, Mainz, Germany).

One panel of WNV RNA-positive plasma samples from clinical cases in the US was provided by L. Tobler from the Blood Systems Research Institute, San Francisco.

Panels of anti-DenV and anti-TBEV positive specimens were designed with materials obtained from the respective clinical cases in Germany, which were kindly provided by Universität München, Abt. für Infektions und Tropenmedizin (T. Löscher), Universität Freiburg, Institut für Medizinische Mikrobiologie & Hygiene (D. Neumann-Haefelin), Universität Heidelberg, Hygiene Institut (P. Schnitzler) and Universität Frankfurt, Zentrum für Hygiene (R. Allwinn).

Aliquots of plasma pools collected for the fractionation of plasma derivatives were obtained from different plasma manufacturers. The respective plasma units had been collected in the US, in Europe or in Asia during the years 2004 and 2005.

ELISA Tests

The following ELISAs were performed strictly following the instructions given in the package insert: "Flavivirus IgG indirect ELISA" (PANBIO Brisbane, Australia), "Anti-West-Nile-Virus-ELISA IgG" (prototype version, Euroimmun, Lübeck, Germany), "West Nile Virus IgG DxSelect™" (Focus Diagnostics, Cypress, CA), and "Enzygnost anti-TBE Virus IgG and IgM" (DADE Behring, Marburg, Germany).

WNV NATs

Human plasma or serum and cell culture-derived samples were tested for WNV RNA using the "Procleix WNV assay" (Chiron Corporation, Blood Testing Division, Emeryville, CA). This test system is a qualitative nucleic acid amplification technique (NAT) based on TMA (transcription mediated amplification) technology. This NAT was performed after passing a training seminar organized by the manufacturer and strictly following the manufacturer's instructions.

WNV-RNA was quantified with the Artus Real Art™ WNV LC RT RCR Kit (QIAGEN, Hilden, Germany) using the QIAamp Viral RNA Mini Kit (QIAGEN) for viral RNA extraction. Quantification standards were provided with the test kit and run in parallel.

Viruses and Cells

Bovine viral diarrhea virus (BVDV), strain Osloss, was obtained from G. Pauli, Robert Koch Institut, Berlin, Germany, and was propagated and titered in MDBK cells (ATTC CCL-22). SFV was provided by J. Thiel (Institut für Virologie, Universität Giessen, Germany), and was propagated and titered in Vero cells (ATTC CCL-81). WNV (1999 New York isolate) was received from T.R. Kreil (Baxter, Vienna, Austria), and was propagated and titered in Vero cells in a biosafety level 3 laboratory.

Virus Titration

Virus infectivity was quantified by estimation of the tissue culture infectious dose ($TCID_{50}$) using standard cell culture conditions. Briefly, Vero and MDBK cells were grown to confluence in 96-well microtiter plates (MTPs). Threefold serial dilutions of the samples in

DMEM medium were prepared, and eight replicates per dilution were assayed by inoculation of a 50 µl sample per well. The cytopathic effects of WNV, Semlilki Forest Virus (SFV), and BVDV were checked on days 3–6 post-infection. The $TCID_{50}$ was calculated according to the Maximum Likelihood statistical tool. If no virus was detected, the limit of detection was calculated according to the Poisson distribution.

WNV Neutralization Assay

For the WNV neutralization assay, 50 µl of a 1:3 serial dilution (1:10 to 1:270) of inactivated (56°C, 30 min) serum was mixed in eight replicates in MTP wells with 50 µl of 20 $TCID_{50}$ units of virus. After incubation for 60 min at 37°C, 50 µl of each well was transferred to another well containing 60% confluent (6,000–7,000) Vero cells. MTPs were incubated for 5–6 days at 37°C, and wells containing evidence of viral cytopathic activity were scored. The log ND_{50} was calculated according to the Maximum Likelihood function [Kundi, 1999]. For a high throughput version of the neutralization assay, only one serum dilution (1:30) was tested under the same conditions described above.

Virus Inactivation Studies

Pasteurization was performed at laboratory scale to assess this virus inactivation step. Stabilizer (1.1 g/ml saccharose, 0.3 g/ml glycine, 0.0162 g/ml $CaCl_2$ dihydrate) was added to 50 ml of a commercial 5% human normal immunoglobulin preparation. The solution was spiked with virus and heated to 60°C. Samples were taken after time intervals as indicated (0–10 hr) and immediately cooled. Cooled samples were subsequently titered. Before application on indicator cells, samples were diluted 1:100 in order to avoid cytotoxic and interfering effects of the test material. Absence of cytotoxic effects was verified by microscopic examination of control cells inoculated with non-spiked 1:100 diluted test material. Absence of interfering effects was verified by positive detection of virus from diluted (1:100) test material that had been spiked with a known amount of virus (100 $TCID_{50}$ per well).

RESULTS

Sensitivity and Specificity of Anti-WNV IgG ELISAs

To assess the relative diagnostic sensitivity and specificity of three anti-WNV IgG ELISAs, we used sera from well-characterized flavivirus-infected patients: 26 WNV-infected, 39 TBEV-infected, and 13 DenV-infected individuals.

Two assays (PANBIO, Focus) recognized all of the 26 anti-WNV-positive sera originating from U.S. patients. The third ELISA, the Euroimmun prototype ELISA version, missed three specimens, resulting in a relative sensitivity of 88% in this study (Table I).

The cross-reactivity rates with related flaviviruses were determined for the anti-WNV ELISAs using specimens from DenV or TBEV-infected patients. The highest cross-reactivity rate (lowest specificity) with these specimens was obtained for the PANBIO assay (92.3% and 79.5%, respectively) followed by the Focus ELISA (92.3% and 56.4%, respectively) and the Euroimmun prototype ELISA, which displayed cross-reactivity rates of 35.7% and 17.9%, respectively.

Based on this analysis of the relative sensitivity and specificity of the different WNV ELISA assays, the following test algorithm for determination of the WNV prevalence was chosen: after screening donor blood with the sensitive Focus ELISA test, a further analysis of all reactive specimens was performed with the more specific Euroimmun prototype WNV ELISA. Those samples that were reactive (positive or borderline) in the second anti-WNV ELISA were then tested in a WNV neutralization assay as a confirmatory test. Additionally, we characterised all samples reactive in the Focus WNV ELISA with an anti-TBEV IgG ELISA. However, this assay displayed 42% cross-reactivity when anti-WNV-positive specimens were tested.

WNV Prevalence

More than 14,000 healthy blood donors from Hesse/Germany were screened for anti-WNV specific IgG antibodies. 5.9% (852/14,437) of the tested donors were reactive in the Focus anti-WNV ELISA test. To estimate the potential impact of TBEV vaccination on the test results, more than 900 blood donors from Austria (where TBEV vaccination is a general public health measure) were also screened for anti-WNV. Seventy-two percent of these blood donors (669/928) were reactive in the anti-WNV ELISA.

All anti-WNV reactive samples from German blood donors were re-tested in the second anti-WNV ELISA and in the anti-TBEV IgG assay. Thirty-four percent of these samples were reactive in the second anti-WNV and 15% were non-reactive in the anti-TBEV ELISA, whereas 9.7% were reactive in the WNV-ELISA and non-reactive in the TBEV-ELISA.

TABLE I. Sensitivity and Cross-Reactivity of Different Anti-WNV IgG ELISAs (pos = Positive; React = Reactive)

		Anti-WNV IgG ELSA		
	Sera	PANBIO	Focus	Euroimmun
Sensitivity	Anti-WNV IgG positive	100% (26/26)	100% (26/26)	88% (23/26)
Cross-reactivity	Anti-dengue IgG positive	92.3% (12/13)	92.3% (12/13)	35.7% (5/14)
	Anti-TBE IgG positive	79.5% (31/39)	56.4% (22/39)	17.9% (7/39)

Two hundred two of the pre-selected anti-WNV suspicious blood donor samples were tested in the WNV neutralization assay. Most of these plasma samples (148/202; 73%) had no or very low WNV neutralization activity (log $ND_{50} < 1$), while 50 specimens (25%) neutralized WNV infection, with log ND_{50} titers between 1 and 2. Only four plasma samples from our blood donors exhibited relatively high neutralization titers (log $ND_{50} \geq 2$) equivalent to those titers observed with the anti-WNV IgG sera from US patients. To check whether any anti-WNV positive might have been missed by our test algorithm, 388 additional anti-WNV reactive specimens were tested in a high throughput neutralization assay. No further specimens with titers of log $ND_{50} > 2$ were identified by this approach.

WNV Incidence

The WNV incidence in a population of healthy German blood donors was investigated during the mosquito season of Summer 2005. In total, 9,976 blood donors were tested for the presence of detectable WNV-RNA using the Procleix WNV NAT assay in minipools of eight. All of the 1,247 minipools tested WNV-RNA negative, with the exception of one initially reactive test result for one minipool. This result was not confirmed on retesting and on testing of the individual plasmas. Furthermore, 198 of the anti-WNV reactive blood donor samples (see above) were tested as individual specimens, with negative test results. Plasma or serum samples collected from a population of German intravenous drug users (IVDU, n = 78), representing a population with increased risk for blood-borne pathogens also tested negative for WNV-RNA (Table II).

Plasma Derivatives

Plasma pools for manufacturing of plasma derivatives with the source plasma collected in the US, East Asia, or Europe were analyzed for the presence of WNV RNA using the Chiron TMA assay. All plasma pools from East Asia (n = 51) and Europe (n = 96) tested negative, while 32 out of 174 (18%) plasma pools from the US tested WNV RNA-positive in this qualitative WNV-NAT (Table II). These pools had been collected in different regions of the US during the years 2004 and 2005. Since the viral load in most pools was too low for accurate results in the quantitative WNV-NAT, viral load was analyzed after concentration of WNV particles by ultracentrifugation and subsequent extraction of the viral nucleic acids. Viral loads were calculated as ranging from 57 to 837 copies WNV-RNA/ml plasma, with 351 copies WNV-RNA/ml as an average value for the TMA-positive pools.

TABLE II. Detection of WNV RNA in Blood Specimens and Plasma Pools Using the Procleix WNV Assay (Chiron)

	Tested	WNV-RNA positive
Blood donors (pools of n = 8)	9,976	0
IVDUs	78	0
Anti-WNV reactive blood donors	198	0
Plasma pools (Europe)	96	0
Plasma pools (USA)	174	32
Plasma pools (East-Asia)	51	0

Virus inactivation steps are included in the manufacturing process of different plasma derivatives. For the production of human-derived medicinal products, a 10 hr heating step of a liquid product intermediate at 60°C (pasteurization) is often performed to inactivate a wide range of potential virus contaminants. The inactivation kinetics of WNV upon pasteurization of a sucrose-stabilized immunoglobulin preparation was compared with the inactivation kinetics of other commonly used model enveloped viruses. BVDV is a pestivirus that frequently serves as a model virus for hepatitis C virus and other members of the Flaviviridae. The inactivation kinetics of WNV were similar to the inactivation kinetics of BVDV. The inactivation kinetics (Fig. 1) confirm that WNV is effectively inactivated by this commonly used manufacturing step.

WNV Reference Preparation

We established and characterized a WNV reference preparation that may be useful for standardization and control of WNV-NATs and WNV-neutralization assays. Supernatant from WNV-infected Vero cells was harvested and characterized for its infectivity titer ($TCID_{50}$) and WNV-RNA content. WNV-RNA concentration was determined by replicate (24 per concentration) testing of limiting dilutions (factor of 2) using the qualitative WNV-NAT followed by calculation of the 95% cut-off concentration using Probit analysis. WNV-RNA concentration was also determined using the quantitative NAT test.

Both NAT approaches revealed a WNV-RNA concentration for the stock material of 6.5×10^9 or 8.1×10^9 copies/ml. Titration in Vero cells gave an infectivity titer of log 7.33 TCID/ml, correlating to approximately 340 copies WNV-RNA per infectious dose.

DISCUSSION

New emerging pathogens may be a threat to public health, not only because of their impact on the population, but also because of their potential to contaminate the blood or plasma supply and to be transmitted to recipients of blood products. Therefore, a research project was initiated by the German Ministry of Health after the huge WNV epidemic that followed the introduction of the virus to the New World in 1999. This research project was performed in cooperation among the Robert-Koch-Institut (RKI; Berlin), the Bernhard-Nocht-Institut (BNI; Hamburg), and the Paul-Ehrlich-Institut (PEI; Langen). In this study, we investigated whether WNV is or could become a threat to public health and the blood supply in Germany. Here, we focus on the prevalence and incidence of WNV among healthy blood donors and the potential for the transmission of the pathogen via plasma derivatives.

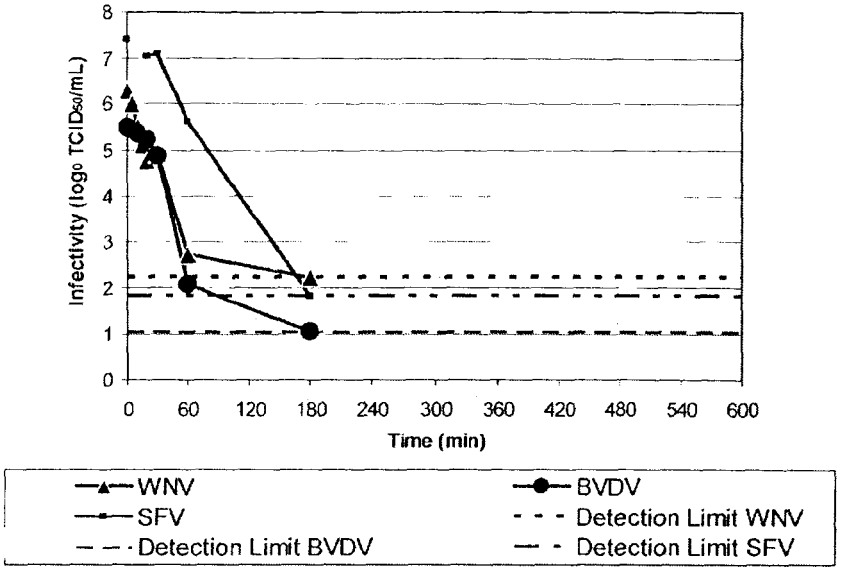

Fig. 1. Virus inactivation through heat inactivation (pasteurization). A sucrose-stabilized human immunoglobulin preparation (5%) was spiked with BVDV, SFV or WNV. After incubation at 60°C, samples were taken after time intervals as indicated (0–10 hr) and cooled immediately. Virus infectivity was quantified by calculation of the tissue culture infectious dose ($TCID_{50}$).

WNV Prevalence

First, we wanted to qualify the anti-WNV screening tests and define an appropriate test algorithm. We decided to use anti-WNV positive specimens from the US as decisive specimens for testing sensitivity for different reasons. First, the respective materials from clinically ill patients were easily available from the US. Second, WNV infections had been confirmed by the clinical course, the seasonal occurrence of the infection and the results of different diagnostic assays performed on individual follow-ups. Furthermore, TBEV, the human flavivirus most frequently found in Europe, is not yet present on the American continent. Therefore, cross-reacting TBEV antibodies should not be an issue for these samples.

The sensitivity was 100% for two anti-WNV assays and 88% for the third assay using such an anti-WNV positive serum panel. However, further panels composed of either anti-Dengue or anti-TBEV positive specimens showed high rates of cross-reactivity with the more sensitive anti-WNV assays and low cross-reactivity with the third assay. We decided to include an anti-TBEV assay as a further diagnostic tool because all of the anti-WNV positive specimens from the US that were missed by the third assay tested as anti-TBE negative, and only a few members of the entire US anti-WNV panel cross-reacted in the anti-TBEV assay. We aimed to preferentially choose anti-WNV reactive/anti-TBEV negative specimens for entering the "gold standard" WNV neutralization test. However, even with this standard, we experienced a high rate of cross-reactivity with anti-DenV-positive specimens. Fortunately, anti-TBEV-positive specimens, which are much more frequent in our region, showed cross-neutralization only at a low level. In conclusion, serological flavivirus diagnostics show high rates of cross-reactivity, and correct interpretation of test results requires extreme caution.

To determine the extent of past WNV infections, samples from more than 14,000 German blood donors were screened with the Focus anti-WNV IgG ELISA. Nearly 6% of the German blood donors were anti-WNV reactive in this assay. Many of the reactive test results were probably caused by cross-reactive antibodies originating from a related flavivirus infection or from vaccination. Though some parts of Southern Germany are TBE risk areas (as the clinical cases show), no reliable TBE incidence or prevalence data are available for Germany.

The TBE vaccination coverage of the Austrian population is in the range of 80%. Sera from Austrian blood donors had a similar reactivity rate in our anti-WNV screening assay. This illustrates the high rate of anti-TBEV cross-reactivity and confirms the similar results obtained in our test qualification study (see above).

Therefore, the anti-WNV reactive rate for blood donors in Hesse (5.9%) may primarily reflect the TBEV vaccination and/or infection level in donors from our region.

To narrow down the number of samples that had to be tested in a WNV neutralization assay, all blood donations that were reactive in the Focus ELISA were re-tested in the more specific prototype anti-WNV IgG ELISA test and in an anti-TBEV IgG assay.

Blood samples that were non-reactive in the anti-TBEV ELISA and reactive in the pre-market anti-WNV

ELISA were tested in the WNV neutralization assay, the "gold standard" for antibody detection, to confirm a past infection. Most of the pre-selected specimens did not inhibit WNV infection in cell culture at all or did so only at a low level (log $TCID_{50} < 1.5$). The titer of 13 additional samples was between ≥ 1.5 and <2.

Only four blood donor samples exhibited a log titer of >2, which was similar to those titers we determined for different clinically confirmed WNV positive sera from the US (Table I). We interviewed the four anti-WNV positive blood donors to evaluate if these putative WNV infections occurred in Germany or Europe or if they might be associated with WNV-endemic countries. One of the four donors had never left Europe, but had visited South and East Europe; another donor had only stayed outside Germany in Turkey. The other two donors had traveled abroad to the USA and Africa. We therefore cannot exclude that singular sporadic and asymptomatic WNV infections may have occurred in Europe and the Middle East. However, WNV prevalence among German blood donors is overall very low; in our study, only 0.03% (95% CI: 0.01–0.07%) of blood donors that were tested were confirmed positive for anti-WNV antibodies by our approach.

WNV-RNA Incidence

For WNV infections, a diagnostic window of only few days (until detection of anti-WNV) has been described during which viral RNA may be detectable. With a WNV-RNA concentration range described from <50 copies/ml up to 10^5 copies/ml [Busch et al., 2005b], the short viremia is at a moderate level when compared to the respective figures for other blood-borne viral infections like HCV (3 months, 10^5–10^8 IU/ml) or HIV (4 weeks, 10^2–10^6 IU/ml) [Lelie et al., 2002].

WNV-RNA incidence in Germany was analyzed in minipools comprised of eight blood donations collected during Summer 2005 and using the highly sensitive Chiron Procleix WNV NAT [Gallian et al., 2005]. Since different WNV strains were described as circulating in Central Europe [Bakonyi et al., 2005, 2006], we investigated whether the Procleix assay detects all available WNV strains. A panel from an external quality assurance study [Niedrig et al., 2006] was retrospectively analyzed. The result confirmed the capability of the Procleix assay to detect WNV lineage 1 and 2 with high sensitivity (data not shown).

No minipools were confirmed positive for WNV-RNA after screening of 1,247 minipools (equal to 9,976 donations). Negative NAT results were also obtained for all individually tested samples from a population that was at increased risk for blood-transmitted infections [i.e., intravenous drug users (IVDUs, n = 78)], or for plasma samples of serologically anti-WNV-reactive blood donors (n = 198).

This outcome is in accordance with the recent WNV-RNA screening results of more than 60,000 Dutch blood donors [Koppelman et al., 2006]. Regardless, blood donors who had just recently visited a WNV endemic country (e.g., the USA) are excluded from the actual donation as a precautionary measure in German blood banks.

Plasma Derivatives

Plasma derivatives (e.g., immunoglobulin preparations, albumin, factor concentrates) may enter the European market with source materials collected in different parts of the world. We were interested in the question of whether WNV-RNA is detectable in aliquots of manufacturing plasma pools.

Some of the plasma pools with plasma of US origin tested positive for WNV RNA by the qualitative NAT assay. The viral load in these plasma pools was determined in the range between 57 and 837 copies/ml, with a median of 351 copies/ml. Each of these pools was composed of several thousand plasma units. Assuming that WNV input in these pools originates from only few donors, WNV concentration of 10^5–10^6 copies/ml are calculated for some individual plasma units. Such high WNV-RNA concentrations have been described for the diagnostic window phase in few cases. However, WNV input into plasma pools from lower viremia cases may be not detected due to the dilution effect though they are expected to be much more frequent and representative for the early infection phase.

Several studies proved that inactivation steps commonly used during the manufacture of plasma derivatives, such as pasteurization for human albumin, S/D treatment for IVIG and FVIII inhibitor-bypassing activity, and incubation at low pH for IVIG, should be sufficient to inactivate enveloped viruses present in source materials [Kreil et al., 2003]. We comparatively investigated the efficacy of pasteurization with regards to WNV and model viruses for enveloped viruses. In these experiments WNV inactivation kinetics were similar between WNV and the two model viruses, BVDV and SFV. This result confirmed the validity of predictions based on model viruses if chosen appropriately. It allowed the conclusion that moderate WNV concentrations in plasma pools should not pose an infection risk to recipients if virus inactivation procedures are included in the manufacturing process of these biological medicinal products.

CONCLUSION

There is currently no indication that WNV could cause an epidemic in Europe similar to that in the USA during the recent years, although temporally and regionally limited outbreaks of WNV infections in humans and horses have been observed in Europe since the 1950s. In contrast to North America, Europe has had direct vector contact with WNV endemic areas in Africa for a long time, via migratory birds, for example. This may have resulted in natural herd immunity in birds. In America, a highly pathogenic WNV strain was imported in 1999 into a virgin territory, meeting a bird population without herd immunity. Environmental factors, such as climate change or global warming and the increasing

mobility of people, may enhance the emergence of new viruses. Therefore, continuous surveillance is an important tool to protect public health and the safety of the blood supply.

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REFERENCES

Allwinn R, Doerr HW, Emmerich P, Schmitz H, Preiser W. 2002. Cross-reactivity in flavivirus serology: New implications of an old finding? Med Microbiol Immunol 190:199–202.

Bakonyi T, Hubalek Z, Rudolf I, Nowotny N. 2005. Novel flavivirus or new lineage of West Nile virus, central Europe. Emerg Infect Dis 11:225–231.

Bakonyi T, Ivanics E, Erdelyi K, Ursu K, Ferenczi E, Weissenbock H, Nowotny N. 2006. Lineage 1 and 2 strains of encephalitic West Nile virus, central Europe. Emerg Infect Dis 12:618–623.

Biggerstaff BJ, Petersen LR. 2002. Estimated risk of West Nile transmission through blood transfusion during an epidemic in Queens, New York City. Transfusion 42:1019–1026.

Busch MP, Caglioti S, Robertson EF, McAuley JD, Tobler LH, Kamel H, Linnen JM, Shyamala V, Tomasulo P, Kleinman SH. 2005. Screening the blood supply for West Nile virus RNA by nucleic acid amplification testing. N Engl J Med 353:460–467.

Busch MP, Tobler LH, Saldana J, Caglioti S, Shyamala V, Liinen JM, Phleps B, Smith RI, Drebot M, Kleinman SH. 2005. Analytical and clinical sensitivity of West Nile virus RNA screening and supplemental assays available in 2003. Transfusion 45:492–499.

Centers for Disease Control and Prevention (CDC). 2006. http://www.cdc.gov/ncidod/dvbid/westnile/index.htm, accessed at October 23, 2006.

Gallian P, De Micco P, de Lamballerie X, Levayer T, Levacon F, Guntz P, Mercier B, Dupond I, Cornillot C, Andreu G. 2005. Prevention of West Nile virus Transmission by blood transfusion: A comparison of NAT Screening assays. Transfusion 45:1540–1541.

Hollinger FB, Kleinman S. 2003. Transfusion transmission of West Nile virus: A merging of historical and contemporary perspectives. Review. Transfusion 43:992–997.

Iwamoto M, Jernigan DB, Guasch A, Trepka MJ, Blackmore CG, Hellinger WC, Pham SM, Zaki S, Lanciotti RS, Lance-Parker SE, DiazGranados CA, Winquist AG, Perlino CA, Wiersma S, Hillyer KL, Goodman JL, Marfin AA, Chamberland ME, Peterson LR. 2003. Transmission of West Nile virus from an organ donor to four transplant recipients. N Engl J Med 348:2196–2203.

Koppelman MH, Sjerps MS, de Waal M, Reesink HW, Cuypers HT. 2006. No evidence of West Nile virus infection in Dutch blood donors. Vox Sang 90:166–169.

Koraka P, Zeller H, Niedrig M, Osterhaus ADME, Groen J. 2002. Reactivity of serum samples from patients with a flavivirus infection measured by immunoflorescence assay and ELISA. Microbes Infec 4:1206–1215.

Kreil TR, Berting A, Kistner O, Kindermann J. 2003. West Nile virus and the safety of plasma derivatives: Verification of high safety margins, and the validity of predictions based on model virus data. Transfusion 43:1023–1028.

Kundi M. 1999. One-hit models for virus inactivation studies. Antiviral Res 41:145–152.

Lanciotti RS, Roehrig JT, Deubel V, Smith J, Parker M, Steele K, Crise B, Volpe KE, Crabtree MB, Scherret JH, Hall RA, MacKenzie JS, Cropp CB, Panigrahy B, Ostlund E, Schmitt B, Malkinson M, Banet C, Weissman J, Komar N, Savage HM, Stone W, McNamara T, Gubler DJ. 1999. Origin of the West Nile virus responsible for an outbreak of encephalitis in the northeastern United States. Science 286:2333–2337.

Lelie PN, van Drimmelen HA, Cuypers HT, Best SJ, Stramer SL, Hyland C, Allain JP, Moncharmont P, Defer C, Nubling M, Glauser A, da Silva Cardoso M, Viret JF, Lankinen MH, Grillner L, Wirthmuller U, Coste J, Schottstedt V, Masecar B, Dax EM. 2002. Sensitivity of HCV RNA and HIV RNA blood screening assays. Transfusion 42:527–536.

MMWR. 2002. Possible West Nile virus transmission to an infant through breast feeding, Michigan. MMWR 51:877–878.

Niedrig M, Linke S, Zeller H, Drosten C. 2006. First international proficiency study on West Nile virus molecular detection. Clin Chem 52:1851–1854.

Petersen LR, Marfin AA. 2002. West Nile virus: A primer for clinician. Ann Intern Med 137:173–179.

Solomon T, Ooi MH, Beasley DW, Mallewa M. 2003. West Nile encephalitis. BMJ 326:865–869.

Stramer SL, Fang CT, Foster GA, Wagner AG, Brodsky JP, Dodd RY. 2005. West Nile virus among blood donors in the United States, 2003 and 2004. N Engl J Med 353:451–459.

Zeller HG, Schuffenecker I. 2004. West Nile Virus. An overview of its spread in Europe and in the Mediterranean Basin in contrast to its spread in the Americas. Eur J Clin Microbiol Infect Dis 23:147–156.

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No. 27

医薬品 研究報告 調査報告書

識別番号・報告回数			報告日	第一報入手日	新医薬品等の区分	機構処理欄
				2007. 12. 4	該当なし	

一般的名称	(製造承認書に記載なし)	研究報告の公表状況	ProMED 20071130-3869, 2007 Nov 30. 情報源:[1]World Health Organisaation (WHO), CSR, Disease Outbreak News, 2007 Nov 30. [2]Agence France Press (AFP) report, 2007 Nov 30.	公表国
販売名(企業名)	合成血「日赤」(日本赤十字社) 照射合成血「日赤」(日本赤十字社) 合成血-LR「日赤」(日本赤十字社) 照射合成血-LR「日赤」(日本赤十字社)			ウガンダ

研究報告の概要

○ウガンダにおけるエボラ出血熱アウトブレイク

[1]ウガンダの保健省は西部のBundibugyo地区でのエボラ出血熱アウトブレイクを確認した。2007年11月28日時点で疑い症例51例、うち死亡例16例が報告されている。報告例には医療関係者の感染も3例含まれており、このうち1例は死亡している。国立研究所および米国疾病対策予防センター(CDC)の実施した臨床検査により、患者検体からエボラウイルスの新種の存在が確認された。現地調査によると、アウトブレイクは2007年9月から始まっていた可能性がある。同国保健省の対策委員会、WHOや他の国際機関は協力して対応に当たっていく。

[2]2007年11月30日、ウガンダ保健省は、同国西部で51人が感染し、少なくとも16人が死亡したエボラウイルスは未知のウイルス株であると発表した。CDCの検査施設に送られた患者の血液及び組織検体を分析したところ、これまでウガンダの他の地区やコンゴ民主共和国で流行していたエボラウイルスの株とは異なった性質が見られた。専門家によると、これまでの株は血管の内膜を破壊することで出血を引き起こし、ショックによって患者を死に至らしめるが、新しい株では出血はそれほど多くなく、患者は高熱を発症後に死亡するとのことである。当局は疫学やウイルス学の専門家を集めて同地区の疾患を監視し、高熱や腹痛、嘔吐、紅斑を発症した人に注意している。

報告企業の意見	今後の対応
ウガンダ西部のBundibugyo地区でエボラ出血熱の集団発生が見られ、エボラウイルスの新種の存在が確認されたとの報告である。	日本赤十字社は、輸血感染症対策として問診時に海外渡航歴の有無を確認し、帰国(入国)後4週間は献血不適としている。今後も引き続き情報の収集に努める。

使用上の注意記載状況・その他参考事項等

合成血「日赤」
照射合成血「日赤」
合成血-LR「日赤」
照射合成血-LR「日赤」

血液を介するウイルス、細菌、原虫等の感染
vCJD等の伝播のリスク

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EBOLA HEMORRHAGIC FEVER - UGANDA: (BUNDIBUGYO), WHO
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A ProMED-mail post
<http://www.promedmail.org>
ProMED-mail is a program of the
International Society for Infectious Diseases
<http://www.isid.org>

[1]
Date: Fri 30 Nov 2007
Source: World Health Organisaation (WHO), CSR, Disease Outbreak News [edited]
<http://www.who.int/csr/don/2007_11_30a/en/index.html>

Ebola haemorrhagic fever in Uganda

The Ministry of Health (MoH), Uganda, has confirmed an outbreak of Ebola haemorrhagic fever, in Bundibugyo District, western Uganda. As of Thu 28 Nov 2007, 51 suspected cases, including 16 deaths have been reported. Among the reported cases, 3 health care workers were also infected, including one fatality. The patients are being hospitalized at Kikyo and Bundibugyo.

Laboratory analysis undertaken at the National Reference Laboratories and the Centres for Disease Control and Prevention (CDC), Atlanta, USA, has confirmed the presence of a new species of Ebola virus in samples taken from cases associated with the outbreak.

Based on initial field investigations, the MoH/WHO Country office has reported that the outbreak might have been ongoing since September 2007. A national task force comprising MoH, WHO and other international partners in the field, is coordinating the response to this outbreak. WHO Country office is assisting the MOH national field team and the District health officials.

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Communicated by:
ProMED-mail Rapporteur Marianne Hopp

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[2]
Date: Fri 30 Nov 2007
Source: Agence France Press (AFP) report [edited]
<http://afp.google.com/article/ALeqM5j8JhykvCqpuWN9lWD4ZjGnYZeA_g>

Uganda's Ebola outbreak is new strain

A lethal Ebola virus that has killed at least 16 people and infected 51 others in western Uganda is a previously unknown strain, health authorities said Friday [30 Nov 2007]. Analysis on victims' blood and tissue samples sent to the Atlanta-based Centers for Disease Control's pathogens laboratory behaved differently from previous known strains of Ebola, they said. "It is a new type of strain. It is different from the one we suffered in Gulu and also different from the one that was reported in the Democratic Republic of Congo," said Sam Okware, who chairs Uganda's national hemorrhagic fever task force. The 1st Ebola case was reported on 10 Nov 2007 in Bundibugyo district on the border with the Democratic Republic of Congo, where 3