医薬品　研究報告 調査報告書

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研究報告の概要	使用上の注意記載状況・その他参考事項等
西ナイルウイルス(WNV) RNA の有無について調べるための 16 人サンプルの「ミニプール」の核酸増幅テスト(NAT)は 2003 年 7 月に開始された。 2003 年 7 月 1 日～10 月 31 日迄の 4 ヵ月間に 677,603 の供血者がミニプールテストによってプロスペクティブにスクリーニングされたところ、183 人が陽性で、検出率は 0.027% (1/3703) であった。 高罹患地域でのミニプールテスト陰性供血者 23,088 検体のレトロスペクティブな個別検査によって、さらに低レベルの WNV 血症 30 検体が検出された。 ウイルス血症ユニットの 5%は IgM 抗体陰性で個別テストによってのみ検出され、29%は IgM 抗体陽転後の個別テストによって発見され、66%はミニプールテストによって検出された。 WNV 感染は、個別テスト陽性で IgM 陰性供血で認められ、抗体陽性で個別テスト陽性の受血者では認められなかった。 供血者のミニプールの核酸増幅テストは 2003 年において、WNV 感染の何百もの症例を防止したが、低レベルウイルス血症のユニットを見逃し、そのいくつかは抗体陰性で、感染性があった。 これらのデータは、2004 年の高い罹患率の地域における個別 NAT 実施の後押しをする。	

報告企業の意見	今後の対応
血漿分画製剤での WNV 感染伝播の報告はなく、製造工程中に WNV と同じフラビウイルスであるウシ下痢症ウイルス(BVDV)の不活化除去が確認された工程を設けているが、今後とも関連情報に注意していく。	今後とも WNV に関連する情報の収集に努めていく。

The NEW ENGLAND JOURNAL of MEDICINE

ORIGINAL ARTICLE

Screening the Blood Supply for West Nile Virus RNA by Nucleic Acid Amplification Testing

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ABSTRACT

BACKGROUND

The use of nucleic acid amplification tests of "minipools" of 16 samples to screen blood donors for West Nile virus RNA began in July 2003. We report the yield and characteristics of positive donations and the incremental yield and safety of nucleic acid amplification tests of individual donations.

METHODS

Reactive minipools were analyzed to identify the individual reactive donations. For the regions with the highest yield on minipool testing, retrospective nucleic acid amplification testing was performed on individual donations that were negative on minipool testing. Reactive donations were confirmed by alternative nucleic acid amplification tests and IgM and IgG tests, and donors were followed to document seroconversion.

RESULTS

From July 1 through October 31, 2003, 677,603 donations were prospectively screened for West Nile virus by minipool testing, yielding 183 confirmed viremic donations (0.027 percent, or 1 in 3703 donations). Retrospective individual testing of 23,088 donations from high-prevalence regions that were negative on minipool testing yielded 30 additional units with a low level of viremia, with 14 additional viremic units detected by prospective testing of individual donations late in the 2003 transmission season. Of all the viremic units detected, 5 percent were detected only by individual testing and were negative for IgM antibody, 29 percent were detected by individual testing after IgM seroconversion, and 66 percent were detected by minipool testing. West Nile virus infection was confirmed in both recipients of IgM-negative units that were reactive on individual testing, whereas neither recipient of antibody-positive blood components that were reactive on individual testing was infected. In 2004, prospective testing of individual donations in regions that yielded donations that were reactive on minipool testing resulted in a 32 percent incremental yield of units with a low level of viremia that would have been missed by minipool testing.

CONCLUSIONS

Although nucleic acid amplification testing of minipools of blood donations prevented hundreds of cases of West Nile virus infection in 2003, it failed to detect units with a low level of viremia, some of which were antibody-negative and infectious. These data support the use of targeted nucleic acid amplification testing of individual donations in high-prevalence regions, a strategy that was implemented successfully in 2004.

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WEST NILE VIRUS, A MOSQUITO-borne flavivirus, emerged as a cause of meningoencephalitis in the United States in 1999, and infections reached epidemic proportions in 2002.[1,2] In 2002, West Nile virus was shown to be transmissible by transfusion, when 23 cases were documented.[3,4] In late 2002, the Food and Drug Administration (FDA), U.S. blood-collecting organizations, and test-kit manufacturers accelerated programs to develop nucleic acid amplification tests to screen blood donors for West Nile viremia in an effort to implement such programs before the 2003 transmission season.[4-7] The resulting assays involved the testing of pools of 6 to 24 samples, or "minipools," an approach that is now also routinely used to screen blood donors for human immunodeficiency virus (HIV) and hepatitis C virus (HCV).[8]

We report the results of a large, multicenter testing program conducted during the summer and fall of 2003 and of studies that compared minipool results with those obtained by nucleic acid amplification tests of individual (undiluted) donations. Our results allow an estimation of the number of infections averted in 2003 by the implementation of screening with minipool nucleic acid amplification tests and an estimation of the additional benefit of screening individual donations in regions with seasonal epidemics of West Nile virus infections. We also summarize experience with implementation of a targeted screening strategy involving nucleic acid amplification testing of individual donations in 2004, a strategy that successfully identified units with a low level of viremia that would have been missed by minipool testing.

METHODS

Blood Systems Laboratories screens blood donations in two FDA-licensed laboratories in Tempe, Arizona, and Bedford, Texas. Clients include 18 blood-collection facilities owned by Blood Systems (Scottsdale, Ariz.) and 59 other community-based and hospital-based blood-collection programs. The geographic areas covered by this client base include much of the Southwest, the South, the Central Plains, and parts of California. The annual testing volume is approximately 2.2 million donations, which represents approximately 20 percent of the U.S. blood supply.

The West Nile virus Transcription-Mediated Amplification system (Procleix WNV Assay, Gen-Probe and Chiron) was used for nucleic acid amplification tests. This technique involves lysis of viral particles in plasma, either from individual donations or from a minipool of plasma specimens from 16 donations and the isolation of West Nile virus RNA with the use of probes bound to magnetic beads, amplification with the use of RNA transcription, and subsequent detection by a chemiluminescent probe.[9,10] All samples within a reactive minipool are then tested individually. The assay has an analytical sensitivity of approximately 4 RNA copies per milliliter when used for individual donations (50 percent limit of detection by probit analysis of dilutions of West Nile virus standards) and a sensitivity of approximately 45 copies per milliliter when used for minipool testing.[10]

Individual specimens identified as reactive were evaluated by means of a confirmatory algorithm with the use of multiple assays for West Nile virus.[10-12] These tests included an alternative nucleic acid amplification test (either a modification of a TaqMan polymerase-chain-reaction [PCR] assay or another primer-based transcription-mediated amplification assay, performed by Bayer Reference Laboratory) and assays of plasma for viral IgM and IgG antibody (Focus Diagnostics).[10,13] All donors with reactive tests were promptly asked to enroll in a follow-up study, involving return visits approximately every week. Follow-up specimens were tested for West Nile virus RNA and for IgM and IgG antibodies against West Nile virus. A confirmed positive result was defined by the detection of viral IgM in either the index specimen or a follow-up specimen, the detection of viral RNA in the index specimen by means of the alternative nucleic acid amplification test, or the detection of viral RNA in a follow-up specimen by means of a transcription-mediated amplification assay.[11] Since the actual times at which donors returned for follow-up varied (resulting in intermittent blood collection) and since seroconversion would have occurred in the interval between the last seronegative and the first seropositive result, median times (and interquartile ranges) to IgM and IgG seroconversion were estimated with the use of an analysis in which data were censored in the intervals between visits.[14] The viral load in confirmed positive index donations for which frozen plasma components were available for analysis was evaluated by a kinetic PCR assay based on target-capture TaqMan techniques (Chiron).[10]

Blood Systems Laboratories also conducted geo-

graphically and temporally targeted nucleic acid amplification testing of individual donations in 2003 according to two protocols. The first protocol involved testing of individual frozen specimens from donations previously found to be negative on nucleic acid amplification testing of minipools of specimens from regions with a high prevalence of West Nile virus infections. Donors whose specimens were retrospectively determined to be reactive on individual testing were asked to enroll in the follow-up study. In addition, in-stock blood products from these donors were retrieved, and in collaboration with the Centers for Disease Control and Prevention (CDC), recipients of blood components from donors confirmed to be positive for West Nile virus on nucleic acid amplification testing of individual donations were evaluated to ascertain whether transmission of West Nile virus had occurred.[15] The second protocol was conducted in selected blood-collection regions that had had a high number of reactive minipools on nucleic acid amplification testing in the previous weeks. Individual donations were prospectively screened exclusively by nucleic acid amplification testing (i.e., minipool testing was not performed). Reactive units were subsequently diluted 1:16 and retested individually to determine what the results of minipool testing would have been. No blood components that were reactive on nucleic acid amplification testing of individual donations were transfused during this prospective study.

In June 2004 Blood Systems Laboratories implemented a targeted screening strategy involving nucleic acid amplification testing of individual donations (described elsewhere in detail[16]) and real-time tracking of the results of minipool testing. Prospective nucleic acid amplification testing of individual donations was implemented in geographically defined zones if there were two or more reactive donations on minipool testing and a rate of more than 1 reactive minipool per 1000 tested. Testing reverted to the minipool format when regions had had no individual donations with reactive tests for at least seven consecutive days and had a weekly rate of reactivity of fewer than 1 per 1000 donations.

All studies were approved by the FDA and the relevant institutional review boards. All donors and recipients gave written informed consent to undergo screening and follow-up testing for West Nile virus. Drs. Busch, Tomasulo, and Kleinman and Ms. Caglioti designed the studies; supervised data collection, management, and analyses; and drafted and revised the manuscript. Drs. Robertson, Tobler, Linnen, and Shyamala and Ms. McAuley supervised testing. Dr. Kamel supervised donor follow-up and participated in "look-back" activities involving recipients of blood components. All authors approved the manuscript, which was written primarily by Drs. Busch and Kleinman.

RESULTS

The results of minipool nucleic acid amplification testing during the active West Nile virus season in 2003 are shown in Figure 1. Of 677,603 donations tested between July 1 and October 31, 2003, 183 were confirmed to be positive, for an aggregate rate of 0.027 percent (1 in 3703 donations). The highest rates occurred during a six-week period from mid-July through mid-August.

Forty-seven additional viremic units were detected on nucleic acid amplification testing of individual donations. Retrospective testing of 23,088 individual donations that had been negative on minipool testing, collected from donor centers in Texas, North Dakota, and South Dakota (i.e., centers with high rates of reactivity on minipool testing during the summer of 2003), identified 30 additional confirmed positive specimens. Prospective testing of individual donations was subsequently performed on 3964 donations collected in North Dakota and South Dakota in September 2003 and identified 17 confirmed positive donations, of which 14 tested negative for West Nile virus with the use of the transcription-mediated amplification system at a 1:16 dilution, indicating they would have been missed by minipool testing. Thus, 186 units were detectable by minipool testing and 44 units were detectable only by nucleic acid amplification testing of individual donations.

West Nile virus antibody status was determined for 41 of the 44 donations that were negative on minipool testing and confirmed positive by individual testing (3 samples had insufficient volume for testing). Thirty-one specimens (76 percent) had detectable West Nile virus antibody: 10 were IgM-positive, and 21 were positive for both IgM and IgG. In contrast, only 16 of the 183 confirmed positive donations detected by minipool testing (9 percent) were positive for IgM antibody at the time of donation ($P<0.001$ by the chi-square test). Among 145 viremic donors who were initially seronegative and who enrolled in the follow-up assessment, West

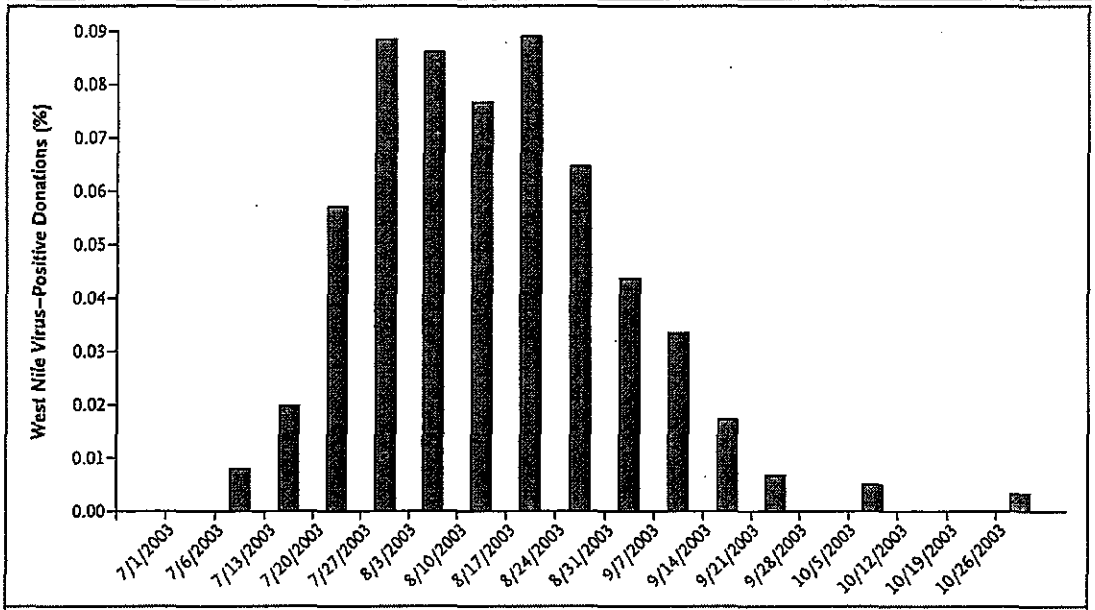

Figure 1. **Results of Minipool Nucleic Acid Amplification Testing during the 2003 West Nile Virus Season.**

Nile virus–specific IgM antibody appeared a median of four days after donation (interquartile range, one to six) and virus-specific IgG antibody appeared a median of two days later (interquartile range, one to five).

The median viral load for 143 of the 183 specimens confirmed to be positive on minipool testing with sufficient volume for quantitative PCR analysis was 3519 copies per milliliter (range, less than 50 to 690,159). Twelve IgM-positive donations identified by minipool testing had significantly lower viral loads than 131 IgM-negative donations identified by minipool testing (median, less than 50 and 5325 copies per milliliter, respectively; $P<0.001$ by the two-sample Wilcoxon rank test). As expected, viral loads were very low in donations detected only by nucleic acid amplification testing of individual donations: 21 of these 44 samples (48 percent) were reactive on only one of two replicate tests, indicating that the viral load was near the limit of detection of the transcription-mediated amplification assay, and 16 of 22 evaluated by TaqMan PCR (73 percent) had RNA levels that were below the limit of quantitation (i.e., fewer than 50 copies per milliliter).

We performed a subanalysis of 113 confirmed viremic donations identified from July 1 through September 30, 2003, in North Dakota and South Dakota from a donor population that had been screened with the use of both minipool and individual nucleic acid amplification testing. Table 1 shows the overall rates of detection of units confirmed positive by minipool testing and by individual testing alone, as well as the incremental rate of detection of viremia by nucleic acid amplification testing of individual donations throughout the epidemic, with adjustment to account for the proportion of units tested individually. Minipool testing detected 66 percent of viremic units detected by nucleic acid amplification testing of individual donations. Five percent of viremic donations were detectable by individual testing alone and were negative for West Nile virus antibody; 7 percent were reactive on individual testing alone and were positive for IgM but negative for IgG; and 22 percent were reactive on individual testing alone and were positive for both IgM and IgG (Table 1).

On the basis of previous studies of inoculation of West Nile virus in humans[17] and animals,[18,19] acute-phase infection is thought to be characterized by a brief period of very-low-level viremia shortly after inoculation (as reflected by the viral-load data presented above and the finding of IgM-negative specimens that were reactive on nucleic acid amplification testing of individual donations). This period is followed by a longer interval (approximately seven days) with an increasing and then decreasing viral load, which makes the viremia de-

Table 1. **Yield of Minipool and Individual Nucleic Acid Amplification Testing of Donations from North Dakota and South Dakota, July 1 through September 30, 2003.**

Variable	Total No. of Donations	Minipool Testing	Individual Nucleic Acid Amplification Testing Alone			
			Total No.	IgM−	IgM+, IgG−	IgM+, IgG+
No. of donations tested	27,009	27,009	22,641	22,641	22,641	22,641
No. of confirmed positive units	113	79	34*	5	6	21
Rate of detection (no. of confirmed positive units/100 donations)	0.443	0.292	0.150	0.024†	0.028†	0.099†
Adjusted no. of confirmed positive units‡	119	79	40	6	8	26
Proportional yield (%)	100	66	34	5	7	22

* Two units had insufficient volume for serologic testing.
† The value was adjusted to account for serologic classification of 32 of 34 units that were identified by individual testing alone.
‡ The value was adjusted to account for individual testing of 22,641 of the 27,009 units that underwent minipool testing.

tectable by minipool testing. Finally, as IgM and IgG seroconversion evolves, the viral load decreases to a level detectable only by testing of individual donations. Given this natural history, Figure 2 presents the biweekly yield data for North Dakota and South Dakota according to the results of individual and minipool tests and antibody tests. The figure shows that although testing of individual donations identified additional donations with a low level of viremia throughout the epidemic, the characteristics of these units shifted from primarily antibody-negative early in the epidemic to predominantly IgM-positive and IgG-positive late in the season ($P<0.001$ by Fisher's exact test).

Case investigations were initiated for 17 recipients of blood components from 14 donations that were negative on minipool testing but identified as having a low level of viremia on retrospective nucleic acid amplification testing of individual donations. On the basis of clinical symptoms and serologic analysis for West Nile virus, two recipients of seronegative donations with low-level viremia were infected with West Nile virus, probably as a result of transfusion, whereas two recipients of components from one donation that was reactive on individual testing alone and was positive for IgM and IgG were not infected.[12] The evaluation of the remaining 13 recipients was deemed inconclusive owing to a lack of follow-up laboratory data to support or rule out West Nile virus infection (Montgomery S and Brown J, CDC: personal communication).

During the 2004 epidemic (from May 1 to October 23, 2004), analysis of 1,065,212 donations by minipool testing yielded 71 confirmed viremic donations (Table 2). An additional 58,679 donations (5 percent of all donations tested) were prospectively tested individually with the use of previously described triggers,[16] and 54 donations were confirmed to be viremic. Sufficient volume was available to test 48 of these 54 donations at a 1:16 dilution: 27 were negative (and thus classified as reactive on individual testing alone), and 21 were positive (and thus classified as detectable by minipool testing). Of the 27 donations identifiable by individual testing alone, 23 were IgM-positive and 4 were IgM-negative. Thus, as seen in Table 2, targeted testing of individual donations in the regions of the 2004 epidemic yielded percentages of units detectable by minipool testing (76 percent) and by individual testing alone (24 percent) and a serologic profile for units detectable by individual testing alone that were similar to the percentages and profile observed in North Dakota and South Dakota during the 2003 epidemic (66 percent and 34 percent, respectively).

DISCUSSION

The implementation of nucleic acid amplification testing for West Nile virus RNA in 2003 resulted in the identification of 183 confirmed viremic units, with 47 additional infected units detected by targeted testing of individual donations. Nationally, the combination of minipool testing and targeted testing of individual donations resulted in the identification of approximately 1000 viremic donations.[11,15] Since, on average, each unit is made into 1.45 transfusable components,[20] the transfusion

of almost 1500 viremic components (most of which lacked antibody and would be expected to be infectious) was averted in 2003.

Our study confirms previous data from clinical cases and experimental inoculation studies indicating that infected persons would probably have low titers of West Nile virus.[2,3,12,15,17-19] Unlike seronegative donors with HIV and HCV infection and positive results on minipool testing, who usually have viral titers of 10^5 to 10^7 copies per milliliter,[6] in our study, the median number of copies of West Nile virus RNA was only about 3500. This observation of a low viral load combined with data indicating that proven transfusion-transmitted cases of West Nile virus infection occurred from donors with low viral titers[3,15] prompted us to study retrospectively the incremental value of individual nucleic acid amplification testing as compared with minipool testing and to implement individual testing prospectively in selected high-prevalence regions in late 2003 and 2004. We found that individual testing identified up to 50 percent more viremic donors than were detected by minipool testing. Five percent of all viremic donations were identifiable by individual testing alone and were antibody-negative and thus were donations that have been shown to be infectious. (Both recipients of units that were antibody-negative and reactive on individual nucleic acid amplification testing alone in our study were infected.) These donations were detected at a fairly constant rate throughout the epidemic. In contrast, the additional yield of antibody-positive donations identified by means of individual testing alone was minimal when tests were performed during the early weeks of the epidemic, but toward the end of the epidemic it increased to levels greater than those observed with minipool testing.

The incremental safety to be achieved by the use of individual testing over minipool testing is difficult to quantify because of the unknown risk of transmission by donations with low-level viremia that contain West Nile virus antibody. The absence of transmission of West Nile virus from two antibody-positive components identified by individual testing alone in this study is consistent with the observation that no documented case of post-transfusion infection has been attributed to a seroreactive donation, despite the relatively high frequency of such donations during the later stages of West Nile virus epidemics.[5,10,15] This is also consistent with in vitro and animal infectivity experiments suggesting that IgM and IgG antibodies neutralize infectivity.[19] Similar experience with hepatitis A virus, an acute infection that also leads to the production of neutralizing IgM antibody, has indicated that the concurrence of viremia and IgM production does not result in infectivity.[7,21] Thus, one working hypothesis is that viremic units that contain IgM (and particularly those that also contain IgG) are not infectious when transfused.

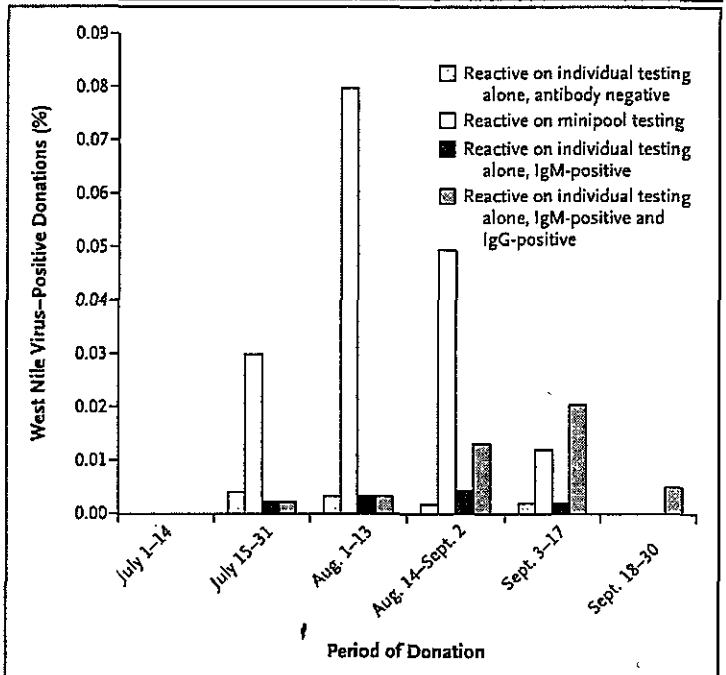

Figure 2. Rate of West Nile Virus–Positive Donations Detected in North Dakota and South Dakota by Minipool and Individual Nucleic Acid Amplification Testing.

The pattern of observed viremia (i.e., the percentage of West Nile virus RNA–positive donations detected by minipool testing as compared with the percentage detected by individual testing) and seroreactivity (presence of IgM and IgG) of viremic units changed dramatically through the 10 weeks of the epidemic, strongly suggesting that the greatest benefit of individual testing can be obtained by implementing such screening early in the epidemic when new infections are on the rise. This observation led Blood Systems Laboratories to adopt a new nucleic acid amplification testing strategy during the 2004 epidemic. A designated level of reactive minipool tests in a defined geographic region was used to determine when sufficient risk existed to implement individual testing.[16] This strategy

Table 2. Actual and Adjusted Systemwide Yield of Minipool and Individual Nucleic Acid Amplification Testing of Donations Screened from May 1 through October 23, 2004.

Type of Nucleic Acid Amplification Test	Total Tested	Confirmed Positive Results		Adjusted Confirmed Positive Results*	
	no. (%)	*no. (%)*	*no./100 donations*	*no. (%)*	*no./100 donations*
Minipool	1,065,212 (95)	71 (57)	0.007	95 (76)	0.009
Individual	58,679 (5)	54 (43)*	0.092	30 (24)	0.051
Total	1,123,891 (100)	125	0.012	125	0.012

* Of the 54 samples confirmed to be positive by nucleic acid amplification testing of individual donations, 48 were available for retesting at a 1:16 dilution; 27 of the latter (56 percent) were found to be negative and therefore classified as reactive on individual testing alone. This percentage was applied to the 54 positive units detected by individual testing to project that 30 of these donations would be positive with the use of this method alone and that 24 would have been detected by minipool testing. The reclassification is reflected in the column that provides adjusted confirmed positive results.

was designed to balance the residual risk of transfusion-transmitted West Nile virus infection accompanying the use of minipool testing against the limited capacity for individual testing, given the current limitations of automation.[5] This strategy proved highly effective, with the identification and removal of at least 27 units that would have been missed by minipool testing in regions with West Nile virus epidemic activity. They included at least four units that were IgM-negative and hence likely to transmit West Nile virus to recipients. Moreover, the similarity of the systemwide distribution of the yields of the two tests in 2004 (an increase in the rate of detection by approximately 32 percent with the use of individual over minipool testing) with that observed in a region of epidemic infection in 2003 indicates that our targeting of individual testing was appropriately directed to regions with an increased yield.

Before the initiation of blood-donor screening, information from experimental studies of the inoculation of West Nile virus conducted in the early 1950s in patients with advanced cancer indicated that the duration of viremia (as assayed by intracerebral injection of virus into mice) was approximately six or seven days.[17] Contemporary data from primate and murine models of West Nile virus infection are consistent with this estimate.[7,18,19] On the basis of the proportional rates of detection of viremia in the early phases of infection in asymptomatic viremic blood donors, we estimate that the duration of antibody-negative viremia detectable only by nucleic acid amplification testing of individual donations is shorter (one or two days). Our data cannot be used to estimate the length of the phase in which specimens are reactive on individual testing and antibody-positive, since a cross-sectional analysis would be biased: the frequency of donation in this convalescent phase is reduced as a result of the signs and symptoms of West Nile virus infection.[2] As compared with other transfusion-transmissible infections (e.g., HIV and HCV), West Nile virus has a similar interval in which it is detectable only by nucleic acid amplification testing of individual donations.[22] In contrast, the duration of viremia detectable by minipool testing is much shorter for West Nile virus infections than it is for HIV and HCV infections, in which high-titer viremia is detected for weeks or months before seroconversion and usually persists for many years after seroconversion.

In conclusion, although the use of minipool screening in 2003 prevented hundreds of West Nile virus infections, it failed to detect donations with a low level of viremia, some of which were antibody-negative and infectious. Our 2003 data supported the use of targeted nucleic acid amplification testing of individual donations in high-prevalence regions, a strategy that was successfully implemented in 2004. On the basis of the price of reagents for previously licensed nucleic acid amplification tests, the costs of performing minipool and individual screening at Blood Systems Laboratories, and the observed yields of minipool testing, the cost of minipool screening was $120,000 per unit intercepted in 2003 and $232,000 per unit intercepted in 2004. The reduced cost utility in 2004 reflects the decreased rate of viremic donations detected, illustrating the close inverse relationship between yield and cost-effectiveness. The targeted individual screening of approximately 60,000 donations in the summer of 2004 resulted in a 33 percent increment in yield and cost only $32,000 per incremen-

tal case detected. In contrast, had individual testing been performed for West Nile virus during all of 2004, the cost per viremic donation detected would have been $281,000. This approach of performing targeted testing of individual donations on the basis of real-time monitoring of the yield of minipool testing may prove to be a rational and cost-effective donor-screening paradigm for other agents similar to West Nile virus that cause seasonal and regional epidemics.

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Dr. Busch is an employee of Blood Systems, a not-for-profit company that collects and tests donated blood for West Nile virus and other infections as described in this article; is a member of Scientific Advisory Boards of Chiron and Gen-Probe, the manufacturers of the West Nile virus assays that were evaluated in this study; and reports having received an unrestricted research grant from Chiron and speaking honoraria from Chiron and Gen-Probe. Ms. Caglioti, Dr. Robertson, Ms. McAuley, Dr. Tobler, Dr. Kamel, and Dr. Tomasulo are employees of Blood Systems. Dr. Linnen is an employee of Gen-Probe and reports owning equity stock and stock options in Gen-Probe. He is named on patents filed by Gen-Probe for the West Nile virus nucleic acid amplification assay described in this article. Dr. Shyamala is an employee of Chiron and is named on patents filed by Chiron for the West Nile virus nucleic acid amplification assay described in this article.

Reagents for performing the retrospective and prospective West Nile virus transcription-mediated amplification testing on collections in 2003 were supplied to Blood Systems at no charge by Gen-Probe. The costs for reagents to perform minipool screening in 2003 and 2004 and individual screening in 2005 were paid to Chiron and Gen-Probe on a cost-reimbursement basis, as detailed in an FDA Investigational New Drug (IND) application. All other sample-collection, shipping, and labor costs related to West Nile virus screening and acquisition of follow-up specimens were funded by Blood Systems, with reimbursement from hospitals or other blood centers. The cost of follow-up serologic and PCR testing was covered by Gen-Probe and Chiron as part of the IND application.

We are indebted to the staff at Blood Systems Laboratories and the United Blood Services and other donor centers for their effort in support of this study and to Susan Montgomery and Jennifer Brown at the CDC for coordination of look-back investigations of recipients exposed to West Nile virus.

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医薬品 研究報告 調査報告書

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研究報告の概要

○フルーツコウモリがエボラウイルスの自然宿主である
ヒトにおけるエボラウイルス流行は1976年に初めて記録されているが、自然宿主は未だにわかっていない。2001年～2003年のガボンおよびコンゴにおけるヒトと大型霊長類のエボラ流行中に、コウモリ679頭、鳥222羽、小型陸生脊椎動物129頭を含む1030頭の動物を捕獲してエボラウイルスの検査を行い、3種類のコウモリにおける不顕性感染の証拠を発見した。
エボラウイルスに特異的なIgG抗体が3種類のコウモリの血清から検出された(ウマヅラコウモリ17頭中4頭、フランケオナシケンショウコウモリ117頭中8頭、クビワフルーツコウモリで58頭中4頭)。エボラウイルスのヌクレオチド配列は肝臓と脾臓からPCRで検出された(ウマヅラコウモリ21頭中4頭、フランケオナシケンショウコウモリ117頭中5頭、クビワフルーツコウモリ141頭中4頭)。睾丸、心臓、肺からウイルスのRNAは検出されず、同じヌクレオチド配列は他の動物からは検出されなかった。
抗体陽性の動物はすべてPCR陰性、PCR陽性の動物はすべて抗体陰性だった。これは、PCR陽性のコウモリは感染から日が浅く、免疫反応が検出できるようになる前に検査されたためであると考えられる。
3種類のコウモリは、ヒトのエボラ流行が起こった地域を含めアフリカに広く生息している。この知見はコウモリがエボラウイルスやマールブルグウイルスなどの自然宿主となっているというこれまでの研究を支持するものである。
他のコウモリや動物種がエボラウイルスの自然宿主となりうるため、ここで取り上げたコウモリの行動の解明が大型霊長類のエボラウイルス感染予防に役立つだろう。現地の人がコウモリを食用としているため、コウモリから人間への直接感染は教育によって予防できると考えられる。

使用上の注意記載状況・その他参考事項等

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血液を原料とすることに由来する感染症伝播等

報告企業の意見	今後の対応
3種類のコウモリにおけるエボラウイルス不顕性感染の証拠を発見したとの報告である。	エボラウイルスは脂質膜を持つ大型RNAウイルスである。これまで、本製剤によるエボラウイルス感染の報告はない。本製剤の製造工程には、平成11年8月30日付医薬発第1047号に沿ったウイルス・プロセスバリデーションによって検証された2つの異なるウイルス除去・不活化工程が含まれていることから、本製剤の安全性は確保されていると考えるが、今後も引き続き、新興・再興感染症の発生状況等に関する情報の収集に努める。

(一千)

BRIEF COMMUNICATIONS

Fruit bats as reservoirs of Ebola virus

Bat species eaten by people in central Africa show evidence of symptomless Ebola infection.

The first recorded human outbreak of Ebola virus was in 1976, but the wild reservoir of this virus is still unknown[1]. Here we test for Ebola in more than a thousand small vertebrates that were collected during Ebola outbreaks in humans and great apes between 2001 and 2003 in Gabon and the Republic of the Congo. We find evidence of asymptomatic infection by Ebola virus in three species of fruit bat, indicating that these animals may be acting as a reservoir for this deadly virus.

Human Ebola outbreaks that occurred between 2001 and 2005 in Gabon and the Republic of the Congo were linked to concurrent outbreaks that devastated local gorilla and chimpanzee populations[2,3]. To identify the viral reservoir, we undertook three trapping expeditions in areas close to infected gorilla and chimpanzee carcasses, just after their discovery (Fig. 1a). In total, 1,030 animals were captured, including 679 bats, 222 birds and 129 small terrestrial vertebrates, and were tested for evidence of infection by Ebola virus (for details, see supplementary information).

Of the infected animals identified during these field collections, immunoglobulin G (IgG) specific for Ebola virus was detected in serum from three different bat species (4 of 17 *Hypsignathus monstrosus*, 8 of 117 *Epomops franqueti* and 4 of 58 *Myonycteris torquata*). Two of the principal organs targeted by Ebola virus are the liver and spleen[4]. Viral nucleotide sequences were detected in these organs in other bats from the same populations (4 of 21, 5 of 117 and 4 of 141, respectively). No viral RNA was detected in kidney, heart or lung in these animals after amplification by polymerase chain reaction (PCR) and no viral nucleotide sequences were revealed in any of the other animal species tested.

Nucleotide-sequence analysis of purified PCR products identified seven different fragments amplified from the 13 PCR-positive animals, all clustering phylogenetically within the Zaire clade (Fig. 1b). The fragments differed not only from one collection to another, but also within a given collection, among the three bat species, and within a given species. The need to use nested PCR indicated that the viral RNA load in tissues was extremely low, which probably explains why we failed to isolate the virus itself.

Surprisingly, none of the IgG-positive animals was PCR-positive, and none of the PCR-positive animals was IgG-positive. This may be because PCR-positive bats were recently infected and were tested before they developed a detectable immune response. Alternatively, it could be that differences in the virulence of Ebola virus strains led to different immunological responsiveness and viral replication patterns. Of the bat species collected at Mbomo in February 2003, 7 of 31 (22.6%) and 0 of 10 (0%) were PCR-positive and IgG-positive, respectively, but five months later the corresponding results were 4 of 184 (2.2%) and 12 of 160 (7.5%). These opposite trends in the PCR and serological results are consistent with the first hypothesis.

Each of the three bat species has a broad geographical range that includes regions of Africa where human Ebola outbreaks occur[5] (Fig. 1c). Our findings support results of previous investigations that identify bats as candidate reservoirs for Ebola and Marburg viruses[1,6], and as reservoirs for the virus families *Paramyxoviridae* and *Rhabdoviridae*[7–9], which are genetically related to Ebola.

Mortality among great apes from Ebola infection can increase during the dry seasons[3] when fruit is scarce in the forest — conditions that foster contact between animals as they compete for food. Immune function in bats also changes during these periods[10], for example as a result of food scarcity or pregnancy, which would favour viral replication and — aided by aggressive interactions — increase infection among great apes. These factors may contribute to the episodic nature of Ebola outbreaks.

Although other bat and animal species may also act as Ebola virus reservoirs, insight into

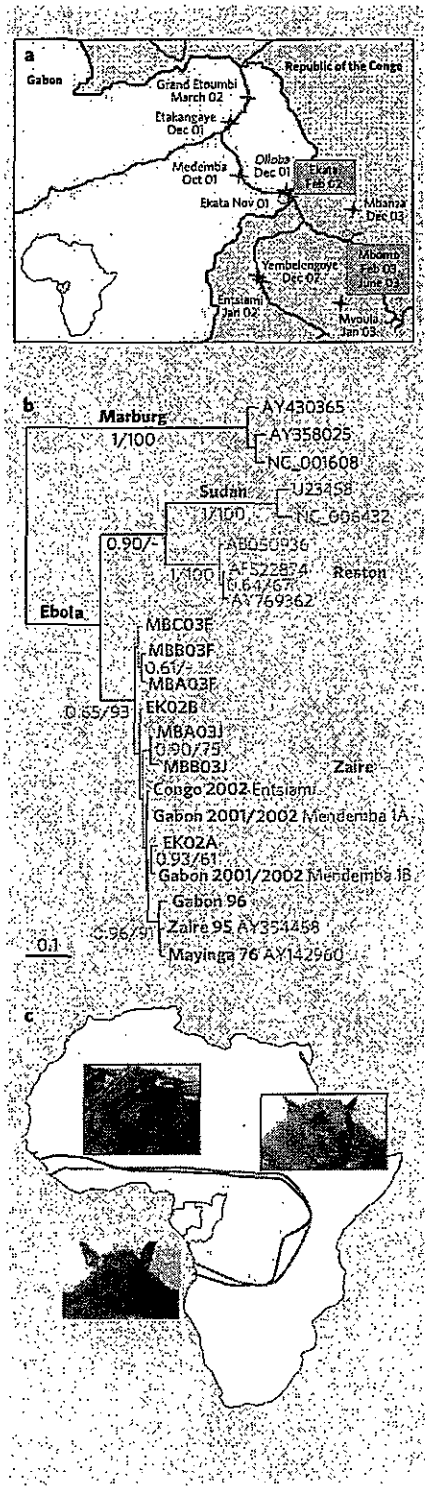

Figure 1 | Fruit bats as potential carriers of Ebola virus. **a**, Dates and locations of animal-trapping sites (blue) and of Ebola virus outbreaks among humans (red stars) in Gabon and the Republic of the Congo. **b**, Phylogeny of Ebola viruses inferred from RNA polymerase sequences. Values below branches are bayesian posterior probabilities (left of slash; values less than 0.5 not shown); bootstrap percentages were obtained by maximum parsimony (right of the slash; values under 50% not shown). (GenBank accession numbers, DQ 205409–205415.) Sequences of the subtype Zaire (red) share five nucleotide signatures in positions 1,755 (T), 1,800 (G), 1,857 (T), 2,002 (A) and 2,003 (C) of the complete coding sequence of the gene encoding RNA polymerase. **c**, Geographic distribution (inside coloured lines) of the fruit bats *Hypsignathus monstrosus* (blue), *Epomops franqueti* (red) and *Myonycteris torquata* (yellow).

the behavioural ecology of the bat species identified here should help to improve protection of the great apes from Ebola virus. Human infection directly from fruit bats might in part be countered by education, as these animals are eaten by local populations living in the outbreak regions.

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PALAEOECOLOGY

A gigantic fossil arthropod trackway

A unique, complex trackway has been discovered in Scotland: it was made roughly 330 million years ago by a huge, six-legged water scorpion that was about 1.6 m long and a metre wide. To my knowledge, this is not only the largest terrestrial trackway of a walking arthropod to be found so far, but is also the first record of locomotion on land for a species of *Hibbertopterus* (Eurypterida). This evidence of lumbering movement indicates that these giant arthropods, now extinct, could survive out of water at a time when the earliest tetrapods were making their transition to the land.

The trackway (Fig. 1a, b) is exposed on a bedding plane close to the base of a sandstone section in a non-marine sequence. It is 6 m long, 0.90–0.98 m wide and consists of sinuous, paired belts of appendage prints flanking a sub-central groove. The trace-maker had at least three pairs of appendages of different lengths (heteropodous), which moved in phase. The longest, outer limbs left elongated crescent-shaped prints (series A in Fig. 1b, green), which overlap slightly or coalesce into a linked series of arcs. The stride length is therefore less than the series-A print length (average, 0.27 m) and indicates that the animal was crawling extremely slowly.

Lines of elongate, crescentic or sigmoidal prints (series B in Fig. 1b, blue) lie inside series A, and further elliptical prints (series C in Fig. 1b, yellow), made by the shortest appendages, can be detected inside these. In places, the series-C prints have been erased by the central groove, which was made by the posterior part of the animal. This is trapezoidal in cross-section and its base is deeper at the margins and slightly raised in the centre. Occasional oblique lineations on the sides and base of the groove indicate that the motion was jerky. The sinuous curve of the groove is smaller in amplitude than, and out of phase with (by about 0.5–0.6 m), the trackway margins, which reveals the direction of locomotion (Fig. 1a, b). The slow, stilted progression, together with the dragging of the posterior, indicates that the animal was not buoyant and that it was probably moving out of water.

There are several groups of Lower Carboniferous (Asbian) arthropods that might have been capable of leaving large trackways[1], but only the water scorpions, or eurypterids[1], are likely to have left the trackway described here. The pattern and character of the limb prints is most consistent with a relatively short-limbed and markedly heteropodous hibbertopteroid eurypterid[2–5] (Fig. 1c). The double-keeled underside of the terminal tail plate of these animals[4,5] matches the character of the central groove.

Fragmentary exoskeletal remains of *Hibbertopterus* and related forms are relatively well known from Scottish Lower Carboniferous rocks[2–5] and were first described from West Lothian in 1831 (ref. 2). The trackway-maker (Fig. 1c) would have been comparable in size to the largest known hibbertopteroid body fossils, which have head shields[3–5] that are 0.65 m wide.

The short length of the relative stride in the trackway emphasizes the extreme slowness of the gait and differentiates it from other eurypterid trackways within the ichnogenus *Palmichnium*[1,6–9]. This trace is 0.2 m (25%) wider than any other trackway of this type[1]. The only larger known invertebrate trackway, although also attributed to a eurypterid, is very different in character and appears to have been made by a swimming animal[10].

Martin A. Whyte

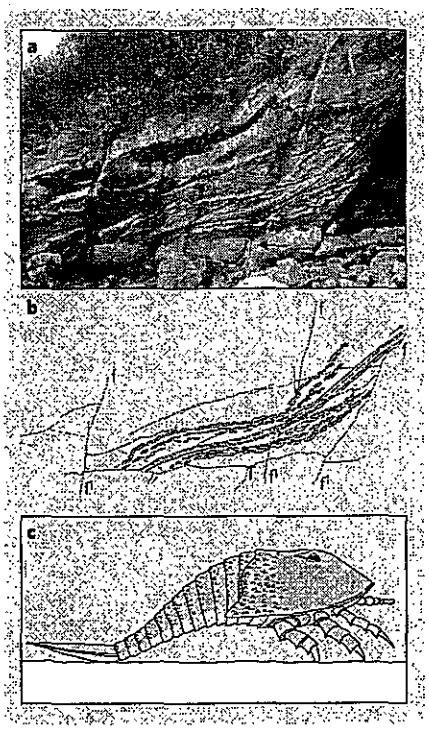

Figure 1 | Hibbertopteroid trackway from Lower Carboniferous (Asbian) rocks in Scotland. **a,** View of the trackway on the undersurface of an overhanging sandstone bed, which is dipping at 45° away from the viewer. The hammer (arrowed) in the photograph is 30 cm long, but the oblique view affects scale and relative proportions. **b,** Interpretive diagram showing track features, position of a second, smaller (0.80 m wide) trackway and the position in the rock of microfaults (f-f'), joints and bedding traces (red lines). Arrow indicates movement direction of the animal. Trackway: orange, central groove; series A, B and C are shown in green, blue and yellow, respectively. **c,** Reconstruction of the hibbertopteroid eurypterid trackway-maker. This arthropod was about 1.6 m. long (for clarity, the limbs on the left of the body are omitted).

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