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Persistent infection with West Nile virus years after initial infection

Kristy Murray, DVM, PhD[1], **Christopher Walker, MPH**[1], **Emily Herrington**[1], **Jessica A. Lewis**[2], **Joseph McCormick, MD**[1], **David W.C. Beasley, PhD**[2], **Robert B. Tesh, MD, MS**[2], and **Susan Fisher-Hoch, MD**[1]

[1]The University of Texas Health Science Center at Houston, School of Public Health, Houston, TX and Brownsville, TX

[2]The University of Texas Medical Branch, Galveston, TX USA

Abstract

West Nile virus (WNV) RNA was demonstrated in 5 of 25 (20%) urine samples collected from convalescent patients 573 to 2,452 days (1.6 to 6.7 years) after WNV infection. Four of the 5 amplicons sequenced showed >99% homology to the WNV NY99 strain. These findings show that individuals with chronic symptoms following WNV infection may have persistent renal infection over several years.

Keywords

West Nile virus; flavivirus; persistent infection; RT-PCR; encephalitis; meningitis

West Nile virus (WNV) is an important flavivirus in North America [1]. Since WNV was first detected in the US in 1999 [2], approximately 25,000 human clinically evident infections have been reported, with more than 1,000 deaths [3,4]. Less than 1% of WNV infected people develop acute neuroinvasive disease, including meningitis, encephalitis, flaccid paralysis, and death [5].

Evidence of persistent WNV infection has been demonstrated in experimentally infected monkeys and hamsters with virus or viral RNA found in brain tissue [6,7]. In addition, hamsters experimentally infected with WNV developed chronic renal infection and shed virus in the urine for up to 8 months [8,9]. Serial urine specimens from these animals contained 10^2 to 10^4 plaque-forming units of infectious virus/ml. Immunohistochemical staining of tissues showed no evidence of WNV antigen in brain, liver, spleen, lungs or bladder, but kidney tissue showed moderate to strong antigen staining. Infectious virus was recovered by co-cultivation of trypsinized fresh kidney cells on Vero cells [9]. These experimental data raise the possibility that persistent renal infection may occur in humans. To date, however, WNV has been

Corresponding author: Dr. Kristy Murray, 1200 Herman Pressler, Houston, TX 77030, (713) 500-9358, (713) 500-9359 fax, Kristy.O.Murray@uth.tmc.edu.

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demonstrated by reverse transcriptase polymerase chain reaction (RT-PCR) only in the urine of a patient with acute WNV infection [10].

When WNV reached Houston in 2002, we established a longitudinal study to follow hospitalized WNV patients to determine risk factors for encephalitis and to understand long-term clinical sequelae. Methods for WNV confirmation and study inclusion criteria were previously described [11]. Informed consent was obtained upon recruitment. This cohort now numbers 112 patients who are evaluated every 6 months, at which time blood is collected and a questionnaire ascertaining subjective and objective measurements of clinical sequelae is completed. At one year post-infection, approximately 60% of patients remain symptomatic, particularly those who were encephalitic. Resolution of symptoms plateaued around two years post-infection, and after 5 years, 60% of patients who presented with encephalitis continued to report clinical symptoms. Chronic symptoms were significantly associated with the persistence of detectable anti-WNV serum IgM ($p=0.026$) and also with a history of hypertension. Cytokine studies showed that many of the chronically symptomatic patients also had significantly elevated plasma levels of interferon gamma inducing protein (IP-10), a marker of active viral infection (K. Murray, unpublished data). There was also evidence of suppression of the Th2 pathway, which might be an indicator of immunosilencing processes facilitating viral persistence. Finally, the deaths of five participants were attributed to chronic renal failure. Collectively, these observations led us to hypothesize that some members of the cohort were persistently infected with WNV and that the kidney could be a preferred site of continued replication and source of shedding. Accordingly, we developed protocols for the collection and processing of urine for the detection of viral nucleic acid using RT-PCR.

Fresh urine was collected from a group of cohort participants into RNAse-free tubes that contained 3.3 units/microliter of Protector RNAse inhibitor (Roche Diagnostics, Indianapolis, IN). An aliquot was immediately extracted and RNA was isolated from convalescent urine according to the manufacturer's protocol for the Qiagen MinElute Virus Spin Kit (Qiagen, Valencia, CA, USA). The urine was not concentrated or pre-treated prior to testing. In addition to standard positive, negative and reagent controls, we included urine from known WNV antibody-negative healthy volunteers to exclude the possibility of amplicon or cross-over contamination during the extraction procedure. Extraction procedures, reactions, and electrophoresis were performed in separate laboratories for the same reason.

Oligonucleotides used for the RT-PCR have been described [12–14]. First-stage primer sequences were 1401: 5'-ACCAACTACTGTGGAGTC-3', and 1845: 5'-TTCCATCTTCACTCTACACT-3', amplifying a 445-bp region of the WNV envelope protein [14]. The amplification reaction was performed according to manufacturer's protocol for the One-Step RT-PCR kit (Qiagen). An aliquot from the first round of PCR was used for the nested PCR with the Taq PCR Core kit. Nested primers used were 1485: 5'-GCCTTCATACACACTAAAG-3' and 1732: 5'-CCAATGCTATCACAGACT-3' for amplifying a 248-bp region [14]. For both the first round and nested PCR, ten μl of each reaction was resolved on an agarose gel (1% w/v), stained with ethidium bromide and visualized with the Gel Bio-Doc-It system for the presence of amplicons. PCR cleanup (Qiagen Quickspin) was used on the first round of PCR product of those specimens which had a positive band detected, and the amplicons were sent to Lone Star Laboratories (Houston, TX) for nucleic acid sequencing.

We tested urine specimens from 25 convalescent patients. RT-PCR was positive in both the first round and nested reactions in five of 25 (20%) urine specimens collected between 573 and 2,452 days post-onset acute clinical disease (see table). Four of the five amplicons from the PCR products from the primary RT-PCR reaction could be sequenced and were found to be >99% homologous to the WNV NY99 strain.

Of the five positive patients, four reported chronic symptoms, including weakness, fatigue, memory loss, and ataxia. All had a clinical presentation of encephalitis, were male, and had a prior history of hypertension. One patient developed kidney failure following his illness. Three patients were more than six years past their initial infection.

Although we suspected viral persistence in some patients with chronic symptoms, finding one in five urine specimens to be positive was unanticipated. For further assurance we repeated the entire RT-PCR procedure in a different location to further exclude the possibility of amplicon contamination. Urine specimens that had been flash frozen in dry ice and ethanol at the time of collection were sent to the University of Texas Medical Branch in Galveston, TX. RNA was extracted using the QIAamp viral RNA mini kit (Qiagen) and then screened by non-nested RT-PCR using oligonucleotide primers targeting a different region of the WNV E gene. Nucleotide sequencing of amplified products confirmed the presence of WNV RNA in samples from two of the patients (2002-42 and 2002-43; Genbank accession numbers GQ495619 and GQ495620, respectively). We are attempting to isolate virus from urine, but thus far have not succeeded. We expected to have difficulty obtaining as isolate since the previous study that identified WNV RNA in urine from an acutely infected human patient was unable to do so [10]. We have also found that the viral RNA degrades quickly in urine, with RT-PCR becoming negative after two or more freeze/thaw cycles. This is a concern; therefore, we are working towards optimizing the testing urine for the presence of viral RNA.

We report here for the first time that WNV is capable of long-term persistence in patients, particularly in the presence of chronic clinical symptoms. The finding of viral RNA in the urine of these patients is suggestive of ongoing viral replication in renal tissue, consistent with the hamster model. The public health impact of these findings is considerable. It will be important to explore and understand the underlying mechanisms related to the shedding of viral RNA in the urine, whether shedding is constant or intermittent, and whether or not this represents true infection resulting in clinically evident disease. Additionally, all five of our positive individuals were older males, and we are unsure at this point if this is a significant finding or simply related to chance. We are currently establishing means to clinically evaluate all cohort participants, particularly with regard to renal function and other abnormalities possibly related to persistent infection with a focus on developing treatment options.

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References

1. Craven RB, Roehrig JT. West Nile Virus. J Am Med Assoc 2001;344:1858–1859.
2. Nash D, Mostashari F, Fine A, et al. The outbreak of West Nile virus infection in the New York City area in 1999. N Engl J Med 2001;344:1807–1814. [PubMed: 11407341]
3. Centers for Disease Control and Prevention. Assessing the capacity for surveillance, prevention, and control of West Nile virus infection—United States, 1999 and 2004. MMWR Morb Mort Wkly Rep 2006;55:150–153.
4. Centers for Disease Control and Prevention. http://www.cdc.gov/ncidod/dvbid/westnile
5. Mostashari F, Bunning ML, Kitsutani PT, et al. Epidemic West Nile encephalitis, New York, 1999: results of a household-based seroepidemiological survey. Lancet 2001;358:261–264. [PubMed: 11498211]

6. Pogodina VV, Frolova MP, Malenko GV, et al. Study on West Nile virus persistence in monkeys. Arch Virol 1983;75:71–86. [PubMed: 6299247]

7. Siddharthan V, Wang H, Motter NE, et al. Persistent West Nile virus associated with a neurological sequela in hamsters identified by motor unit number estimation. J Virol. 2009 Feb 18;[Epub ahead of print]

8. Tesh RB, Siirin M, Guzman H, et al. Persistent West Nile virus infection in the golden hamster: studies on its mechanism and possible implications for other flavivirus infections. J Infect Dis 2005;192:287–295. [PubMed: 15962223]

9. Tonry JH, Xiao S-Y, Siirin M, Chen H, Travassos APA, Tesh RB. Persistent shedding of West Nile virus in the urine of experimentally infected hamsters. Am J Trop Med Hyg 2005;73:320–324. [PubMed: 15772329]

10. Tonry JH, Brown CB, Cropp CB, et al. West Nile virus detection in urine. Emerg Infect Dis 2005;11:1294–1296. [PubMed: 16102323]

11. Murray K, Baraniuk S, Resnick M, et al. Risk factors for encephalitis and death from West Nile virus infection. Epidemiol Infect 2006;134:1325–1332. [PubMed: 16672108]

12. Lanciotti RS, Kerst AJ, Nasci RS, et al. Rapid detection of West Nile virus from human clinical specimens, field-collected mosquitoes, and avian samples by a TaqMan reverse transcriptase-PCR assay. J Clin Microbiol 2000;38:4066–4071. [PubMed: 11060069]

13. Shi PY, Kauffman EG, Ren P, et al. High-throughput detection of West Nile Virus RNA. J Clin Micro 2001;39:1264–1271.

14. Johnson DJ, Ostlund EN, Pedersen DD, Schmitt BJ. Detection of North American West Nile virus by a reverse-transcription nested polymerase chain reaction assay. Emerg Infect Dis 2001;4:739–741. [PubMed: 11585541]

Table

Description of the subset of WNV cohort participants whose urine tested positive by RT-PCR for WNV nucleic acid.

Patient ID	Clinical Presentation of acute WNV	Gender	Age at onset	Days (years) between onset and urine collection	HTN*	Chronic renal disease	Chronic symptoms	Detectable IgM at time of urine collection	Detectable IgM at one year post-onset
2002-015	Encephalitis	Male	45	2,422 (6.6)	Yes	No	Yes	No	No
2002-042	Encephalitis	Male	74	2,442 (6.7)	Yes	Yes	Yes	No	Yes
2002-043	Encephalitis	Male	74	2,452 (6.7)	Yes	No	Yes	No	Yes
2004-016	Encephalitis	Male	78	1,581 (4.3)	Yes	No	No	No	No
2007-003	Encephalitis	Male	68	573 (1.6)	Yes	No	Yes	No	No

*Patient had a history of hypertension prior to WNV infection.