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Rapid Detection of West Nile Virus from Human Clinical Specimens, Field-Collected Mosquitoes, and Avian Samples by a TaqMan Reverse Transcriptase-PCR Assay

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The authors report on the development and application of a rapid TaqMan assay for the detection of West Nile (WN) virus in a variety of human clinical specimens and field-collected specimens. Oligonucleotide primers and FAM- and TAMRA-labeled WN virus-specific probes were designed by using the nucleotide sequence of the New York 1999 WN virus isolate. The TaqMan assay was compared to a traditional reverse transcriptase (RT)-PCR assay and to virus isolation in Vero cells with a large number (≈500) of specimens obtained from humans (serum, cerebrospinal fluid, and brain tissue), field-collected mosquitoes, and avian tissue samples. The TaqMan assay was specific for WN virus and demonstrated a greater sensitivity than the traditional RT-PCR method and correctly identified WN virus in 100% of the culture-positive mosquito pools and 98% of the culture-positive avian tissue samples. The assay should be of utility in the diagnostic laboratory to complement existing human diagnostic testing and as a tool to conduct WN virus surveillance in the United States.

West Nile (WN) virus is an arthropod-borne virus that is taxonomically classified within the family *Flaviviridae*, genus *Flavivirus*, and that is a member of the Japanese encephalitis (JE) virus serocomplex, which includes JE virus, St. Louis encephalitis (SLE) virus, and Murray Valley encephalitis virus (11). The virus possesses a single-stranded plus-sense RNA genome of approximately 11,000 nucleotides. WN virus circulates in natural transmission cycles involving primarily *Culex* species mosquitoes and birds, and humans are incidental hosts. Although human infections in areas of endemicity are common, such infections are usually mild or subclinical. Severe disease is commonly associated with the elderly (4). WN virus infection can also cause mortality among equines as well as among certain domestic and wild birds (6).

Historically, WN virus has circulated primarily in Africa, Asia, southern Europe, and Australia and has been responsible for several significant epidemics, notably, in Israel (1950s), France (1962), South Africa (1974), and Romania (1996) (6, 13, 14, 17). In 1999 WN virus was responsible for two epidemics. One occurred in Volgograd, Russia, and the other occurred in the New York City area, where there were 62 confirmed human cases, with six fatalities (1, 3, 7, 13).

In the diagnostic laboratory, human WN virus infection can be inferred by immunoglobulin M (IgM) capture enzyme-linked immunosorbent assay (ELISA); however, the assay cannot readily differentiate between WN virus, SLE virus, JE virus, and other members of this serocomplex (9). Serologic confirmation of WN virus infection in humans is possible only through detection of the presence of WN virus-specific neutralizing antibody in either cerebrospinal fluid (CSF) or serum by the plaque reduction neutralization (PRNT) assay. Virus isolation in cell culture from CSF, serum, or tissues, followed by virus identification in an immunofluorescence assay with WN virus-specific monoclonal antibodies can also yield unambiguous results. However, both PRNT and virus isolation assays require up to a week for completion, and isolation requires viable virus in samples. Virus isolation in cell culture is also the current method of choice for the detection of WN virus in field-collected mosquito pools and vertebrate tissues.

Reverse transcriptase (RT)-PCR has been used to develop highly sensitive and specific assays for the identification of several RNA viruses, including WN virus (12). Primers for this WN virus RT-PCR assay, however, were designed by using the published sequence of the WN virus Uganda 1939 strain, which demonstrates only 79% similarity to the recent NY99 isolate, resulting in six mismatches in the downstream primer (12). Recently, several diagnostic assays using fluorescent DNA probes in a 5′ exonuclease assay (TaqMan) have been developed for a variety of pathogens. These TaqMan detection assays offer the advantage over traditional RT-PCR of increased sensitivity, higher throughput, increased reproducibility, and better quantitation (5, 8, 10, 16). In order to develop the most sensitive and specific assay for the WN virus NY99 strain, RT-PCR and TaqMan primers were designed on the basis of the genome sequence of the NY99 strain of WN virus. The authors report on the development and extensive laboratory testing of a TaqMan assay and a traditional RT-PCR-based diagnostic assay for the detection of WN virus in a variety of clinical specimens that include human serum, CSF, brain tissue, mosquito pools, and avian tissues.

MATERIALS AND METHODS

Virus strains. All virus strains were obtained from the reference collection maintained at the Division of Vector-Borne Infectious Diseases, Centers for Disease Control and Prevention (CDC). WN virus strain NY99 (flamingo 382-99) was titrated in Vero cells by a standard plaque assay.

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TABLE 1. Oligonucleotide primers and probes used in the RT-PCR and TaqMan assay

Primer	Genome position[a]	Sequence (5′-3′)	RT-PCR product size (bp)
WN233	233–257	TTGTGTTGGCTCTCTTGGCGTTCTT	408
WN640c	640–616	CAGCCGACAGCACTGGACATTCATA	
WN3′NC-forward	10,668–10,684	CAGACCACGCTACGGCG	103
WN3′NC-reverse	10,770–10,756	CTAGGGCCGCGTGGG	
WN3′NC-probe	10,691–10,714	TCTGCGGAGAGTGCAGTCTGCGAT	
WNENV-forward	1160–1180	TCAGCGATCTCTCCACCAAAG	70
WNENV-reverse	1209–1229	GGGTCAGCACGTTTGTCATTG	
WNENV-probe	1186–1207	TGCCCGACCATGGGAGAAGCTC	

[a] Genome position according to WN virus NY99 complete genome sequence (7).

Plaque assay detection of WN virus in mosquito pools and avian tissue. Adult mosquitoes were sorted by species and sex, placed in pools of not more than 50, and stored at −80°C until tested for the presence of virus. Pools of mosquitoes were placed in polypropylene, round-bottom, snap-top tubes (12 by 75 mm; 5 ml; Falcon 352063; Becton Dickinson Labware, Franklin Lakes, N.J.) with 2 ml of BA-1 diluent (1× medium 199 with Hanks' balanced salt solution, 0.05 M Tris buffer [pH 7.6], 1% bovine serum albumin, 0.35 g of sodium bicarbonate per liter, 100 μg of streptomycin per liter, 1 μg of amphotericin B [Fungizone] per ml). Pools were ground by placing four, 4.5-mm-diameter, copper-clad steel beads (BB-caliber airgun shot) into the tube with the mosquitoes and diluent and vortexing on a laboratory mixer for 20 to 30 s. The homogenate was centrifuged in Eppendorf tubes at 14,000 rpm for 3 min to remove the suspended solids, without removing the beads. Alternatively, mosquito pools were triturated in 2 ml of BA-1 diluent with cold mortars and pestles, and the suspensions were centrifuged as described above. Avian tissues were homogenized in Ten Broeck tissue grinders with 2 ml of BA-1 as the diluent and were clarified by centrifugation. The supernatant obtained from both the mosquito pool and the tissue-grinding protocols were tested for virus by using a Vero cell culture plaque assay in six-well plates (2).

To evaluate the sensitivity of the TaqMan and RT-PCR assays for mosquito pool specimens, dilutions of a WN virus-positive mosquito pool were prepared (see Table 2). A single *Aedes albopictus* female mosquito (Keystone strain) was intrathoracically inoculated with WN virus NY99 and was incubated at 27°C for 6 days. The negative mosquitoes were *Aedes aegypti* (DQ strain) from a laboratory colony. The virus-positive pool was ground in 2.0 ml of BA-1 diluent as described above. Serial, 10-fold dilutions of the virus-positive mosquito suspension were made by using the mosquito homogenate as a diluent (50 negative *A. aegypti* mosquitoes ground in 2.0 ml of BA-1 diluent).

RNA extraction, RT-PCR, and TaqMan assay. Viral RNA was isolated from virus seeds, mosquito pools, CSF, serum, and homogenized tissues (avian and human) by using the QIAamp viral RNA kit (QIAGEN, Valencia, Calif.). Mosquito pools and tissues were first homogenized as described above, and total RNA was extracted from 140 μl of the same supernatant used for Vero cell inoculation. RNA was eluted from the QIAgen columns in a final volume of 100 μl of elution buffer and was stored at −70°C until used. WN virus RT-PCR primers were designed from the published sequence of the NY99 strain (GenBank accession number AF196835) with the OLIGO primer design software program (Molecular Biology Insights Inc., Cascade, Colo.) (Table 1). The WN virus TaqMan primers and probe were designed with the PrimerExpress software package (PE Applied Biosystems, Foster City, Calif.). The TaqMan probes were labeled at the 5′ end with the FAM reporter dye and were labeled at the 3′ end with the quencher dye TAMRA. The RT-PCR was performed with the TITAN One-Tube RT-PCR kit (Roche Molecular Biochemicals, Indianapolis, Ind.) by using 5 μl of RNA and 50 pmol of each primer in a 50-μl total reaction volume by following the manufacturer's protocol with the following cycling times and temperatures: 1 cycle of 45°C for 1 h and 94°C for 3 min and 40 cycles of 94°C for 30 s, 55°C for 1 min, and 68°C for 3 min. After the RT-PCR was performed, a 5-μl portion was analyzed by agarose gel electrophoresis on a 3% NuSieve 3:1 agarose gel (FMC Bioproducts, Rockland, Maine) and the DNA was visualized by ethidium bromide staining.

For the TaqMan assay, 5 μl of RNA was combined with 50 pmol of each primer and 10 pmol of the FAM- and TAMRA-labeled probe in a 50-μl total reaction volume by using the TaqMan RT-PCR Ready-Mix Kit (PE Applied Biosystems). The samples were subjected to 45 cycles of amplification in an ABI Prism 7700 Sequence Detection System instrument (PE Applied Biosystems) according to the manufacturer's protocol for TaqMan assay RT-PCR cycling conditions. The rate of TaqMan assay positivity was calculated by using the 99.9% confidence level settings in the PE 7700 Sequence Detection System software. Quantitation of WN virus in human CSF specimens was calculated by generating a standard curve (correlation coefficient, 0.996) with previously titrated WN virus seed by using the PE 7700 Sequence Detection System Software.

Clinical specimens. Twenty-five human brain tissue specimens were obtained at autopsies of patients with suspected cases of viral encephalitis. The autopsies were performed by the New York City Office of the Chief Medical Examiner from August to October 1999. The brain tissue specimens were homogenized as described above for the avian tissues. Fifty-eight CSF specimens and 47 serum specimens were obtained from patients presenting with fever and/or viral encephalitis during the time frame of the WN virus epidemic in New York City. WN virus "serology-positive" human cases were defined by a positive IgM capture ELISA and the presence of detectable WN virus-specific neutralizing antibody, as measured by the plaque reduction neutralization assay.

RESULTS

Sensitivity and specificity of TaqMan and RT-PCR assays. The sensitivities of the TaqMan and the RT-PCR assays were first evaluated by testing 10-fold dilutions of seed WN virus NY99 that had previously been quantitated by plaque titration (Fig. 1; Table 2). Both TaqMan assay primer-probe combinations detected less than 1 PFU of virus, whereas the RT-PCR assay was 10-fold less sensitive, detecting 1 PFU of virus. The sensitivities of the two assays were also compared by testing blindly coded specimens containing dilutions of WN virus in a fixed quantity of uninfected mosquitoes. These specimens were tested in parallel by the standard plaque assay (Table 2). The TaqMan assay Env primer-probe yielded positive results for samples that produced 1 plaque in Vero cell culture, and the 3′NC primer-probe yielded positive results for samples that yielded 10 plaques. The RT-PCR assay was approximately 1,000-fold less sensitive than the TaqMan assay for the detection of virus in mosquito pools.

The primer pairs were tested for specificity by performing the TaqMan and RT-PCR assays with viral RNA extracted from six Old World WN virus strains (strains Egypt 1951, Italy 1998, Romania 1996-H, Romania 1996-M, Kenya 1998, and Kunjin 1960), six serologically related flaviviruses (JE virus, Murray Valley encephalitis virus, SLE virus, dengue virus type 2, yellow fever virus, and Powassan virus), and three arthropod-borne viruses that circulate in North America (eastern equine encephalitis virus, western equine encephalitis virus, and LaCrosse virus). The TaqMan primers-probes were highly specific for WN virus strains; no fluorescent signal was generated with any of the arthropod-borne flaviviruses or other North American arthropod-borne viruses. The 3′NC TaqMan assay primers generated positive results for all of the WN virus strains tested, whereas the ENV primers detected only four of the seven strains tested (strains NY 1999, Romania 1996-M, Italy 1998, and Kenya 1998; Table 2). The RT-PCR primers (WN233 and WN640c) detected all of the WN virus strains but did not yield amplification products with any of the other viruses with the exception of JE virus, with which a DNA band of approximately 400 bp was observed.

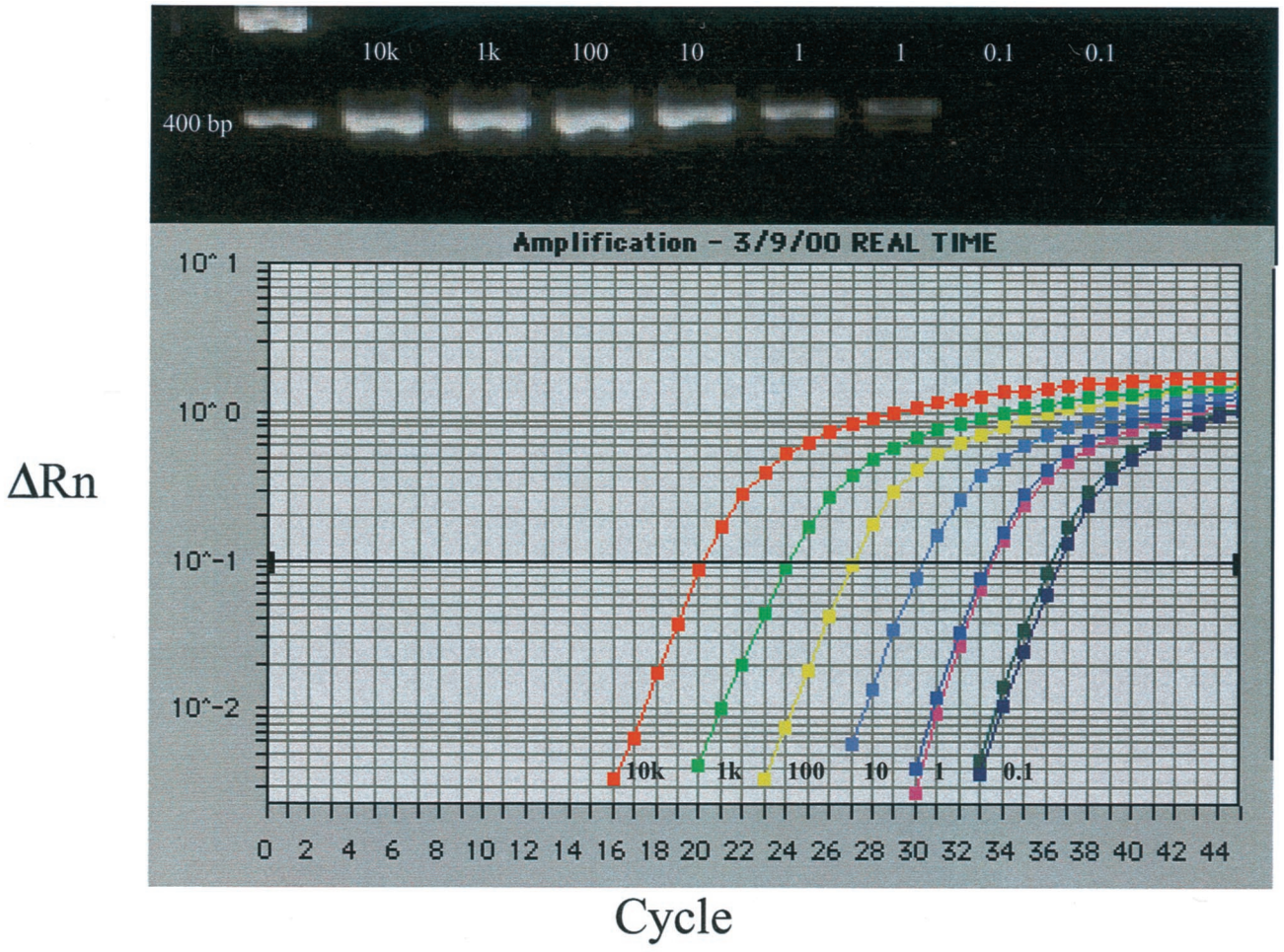

FIG. 1. Sensitivity comparison of the TaqMan and RT-PCR assays for WN virus. The amplification plot was obtained from TaqMan assay testing of previously titrated WN virus dilutions (10,000 to 0.1 PFU; tests with 1 and 0.1 PFU were performed in duplicate) with the 3′NC primer-probe set. The inset in the upper portion depicts agarose gel electrophoresis of the RT-PCR products obtained from the same dilution series of WN virus with the 233-640c primer pair. ΔRn, change above threshold fluorescence.

Detection of WN virus in field-collected mosquito pools and avian tissues. One hundred sixty-five mosquito pool specimens obtained from collections of mosquitoes collected in New York and New Jersey during the WN virus epidemic (September to November 1999) were tested by virus isolation in Vero cell culture, the TaqMan assay, and the RT-PCR assay. WN virus was isolated from 15 of the pools (Table 3), and these same 15 pools (100%) were also positive by the TaqMan assay. Fourteen of the 15 WN virus-positive pools (93%) were also positive by the traditional RT-PCR assay. One hundred ninety-eight avian tissues obtained from dead birds collected during the epidemic were similarly tested by virus isolation in Vero cell cultures, the TaqMan assay, and the RT-PCR assay. WN virus was isolated from 98 of the tissues. Ninety-six (98%) of these were positive by the TaqMan assay, and 91 (93%) were positive by the RT-PCR assay.

Detection of WN virus in human specimens. Human specimens (brain tissue, CSF, and serum) from patients either classified as non-WN virus infected or confirmed to have WN virus infection, determined by serological testing (IgM ELISA and PRNT assay), were tested by the TaqMan and RT-PCR assays. Virus isolation was performed for most of these specimens, and no WN virus was isolated (data not shown). All six of the brain tissues obtained at autopsy from patients with confirmed WN virus infection were positive by the TaqMan assay, and five of these were also positive by the RT-PCR assay (Table 4). Sixteen of the 28 CSF samples from patients with serologically confirmed WN virus infection were positive by the TaqMan assay, and none of these were positive by the RT-PCR assay (Fig. 2). Finally, only 4 of the 28 serum samples from patients with serologically confirmed WN virus infection were positive by the TaqMan assay, and none were positive by the RT-PCR assay.

DISCUSSION

This report describes the development of a rapid TaqMan-based assay for the detection of WN viral RNA in human specimens, mosquito pools, and avian tissue specimens. The assay is highly sensitive and specific and can be performed in as little as 3 h. Compared to the traditional RT-PCR assay, the detection limits of the two systems with WN virus seed are within a factor of 10: both assays detected 1 PFU, and the TaqMan assay detected 0.1 PFU. In contrast, the TaqMan assay demonstrated a marked increase in sensitivity compared to that of RT-PCR when they were applied to the testing of WN virus-infected mosquito pools: 1 to 10 PFU for the TaqMan assay and >100 PFU for the RT-PCR assay. Inhibition of

TABLE 2. Sensitivity and specificity of the TaqMan and RT-PCR assays

Sample (dilution)	Quantity	TaqMan assay[a]						RT-PCR assay result
		3′NC probe			ENV probe			
		Ct	Rn	Int	Ct	Rn	Int	
Titrated seed WN virus								
NY99 (undiluted)	10,000 PFU	20.08	4.11	Pos	18.51	1.82	Pos	Pos
NY99 (10^{-1})	1,000 PFU	24.01	3.85	Pos	22.26	1.71	Pos	Pos
NY99 (10^{-2})	100 PFU	27.06	3.41	Pos	25.74	1.63	Pos	Pos
NY99 (10^{-3})	10 PFU	30.21	3.05	Pos	29.08	1.57	Pos	Pos
NY99 (10^{-4})	1 PFU	34.28	2.35	Pos	32.23	1.41	Pos	Pos
NY99 (10^{-4})	1 PFU	33.1	2.62	Pos	32.55	1.44	Pos	Pos
NY99 (10^{-5})	0.1 PFU	36.18	1.73	Pos	35.69	1.14	Pos	Neg
NY99 (10^{-5})	0.1 PFU	36.43	1.37	Pos	35.82	1.11	Pos	Neg
NY99 (10^{-6})	0.01 PFU	45	0.28	Neg	45.00	0.22	Neg	Neg
NY99 (10^{-6})	0.01 PFU	45	0.3	Neg	45.00	0.19	Neg	Neg
WN virus mosquito pools								
Undiluted	TNTC[b]	19.07	3.9	Pos	22.50	2.11	Pos	Pos
NY99 (10^{-1})	TNTC	22.23	3.61	Pos	23.88	2.20	Pos	Pos
NY99 (10^{-2})	TNTC	27.22	2.69	Pos	29.25	1.83	Pos	Pos
NY99 (10^{-3})	TNTC	28.49	2.36	Pos	30.35	1.75	Pos	Pos
NY99 (10^{-4})	22 plaques	31.15	1.53	Pos	33.10	1.53	Pos	Neg
NY99 (10^{-5})	8 plaques	32.95	1.04	Pos	34.68	1.24	Pos	Neg
NY99 (10^{-6})	10 plaques	33.55	0.89	Pos	34.74	1.31	Pos	Neg
NY99 (10^{-7})	1 plaque	38.7	0.41	Neg	36.87	0.87	Pos	Neg
NY99 (10^{-8})	1 plaque	36.8	0.48	Neg	36.34	0.93	Pos	Neg
NY99 (10^{-9})	0 plaque	38.7	0.44	Neg	45.00	0.29	Neg	Neg
WN virus strains								
Romania 1996-H	ND[c]	24.63	1.66	Pos	45.00	0.24	Neg	Pos
Romania 1996-M	ND	29.02	1.25	Pos	26.04	0.98	Pos	Pos
Egypt 1951	ND	25.54	1.63	Pos	45.00	0.14	Neg	Pos
Italy 1998	ND	23.82	1.52	Pos	23.97	0.89	Pos	Pos
Kenya 1998	ND	21.38	1.75	Pos	21.68	0.88	Pos	Pos
Kunjin 1960	ND	20.58	1.49	Pos	45.00	0.23	Neg	Pos
Other viruses								
Dengue virus type 2	ND	45.00	0.39	Neg	45.00	0.29	Neg	Neg
Yellow fever virus	ND	45.00	0.47	Neg	45.00	0.19	Neg	Neg
SLE virus	ND	45.00	0.43	Neg	45.00	0.17	Neg	Neg
JE virus	ND	45.00	0.42	Neg	45.00	0.29	Neg	Pos
Murrey Valley encephalitis virus	ND	45.00	0.35	Neg	45.00	0.18	Neg	Neg
Eastern equine encephalitis virus	ND	45.00	0.42	Neg	45.00	0.28	Neg	Neg
Western equine encephalitis virus	ND	45.00	0.46	Neg	45.00	0.23	Neg	Neg
Powassan virus	ND	45.00	0.43	Neg	45.00	0.19	Neg	Neg
Lacrosse virus	ND	45.00	0.42	Neg	45.00	0.20	Neg	Neg

[a] Ct, threshold cycle number, which is the cycle number at which fluorescence increases above a fixed threshold value; Rn, normalized fluorescent signal, which is the fluorescent signal generated by the reporter dye. For the 3′NC probe, a threshold cycle number (Ct) of <37 and a normalized fluorescent signal (Rn) of >0.88 is equal to a TaqMan positive interpretation (Int.). For the ENV probe; a threshold cycle number (Ct) of <37 and a normalized fluorescent signal (Rn) of >0.65 is equal to a TaqMan positive interpretation (Int.); Pos, positive; Neg, negative.

[b] TNTC, complete cell death (plaques too numerous to count).

[c] ND, not determined.

the RT-PCR assay, and hence reduced sensitivity, has been observed in our laboratory when the traditional RT-PCR assay is performed with mosquito pools. This inhibition may be due to the large quantity of protein and lipid present in the mosquito homogenate which could inhibit either the RT or the *Taq* polymerase enzyme. The TaqMan assay amplifies much smaller DNA fragments (typically, less than 100 bp), and the fluorescent signal is generated via a 5′ exonuclease probe cleavage mechanism which does not require the synthesis of full-length DNA products. Both of these properties of the TaqMan assay could explain the greater sensitivity of the TaqMan assay compared to that of the traditional RT-PCR assay, in which the amplification of full-length products is essential. An attempt was made to increase the sensitivity of the traditional RT-PCR assay by using primer pairs that amplify much smaller regions (100 to 150 bp); however, the detection limit in tests with WN virus seeds, mosquito pools, and human CSF samples remained essentially unchanged (data not shown).

TABLE 3. Detection of WN virus in mosquito pools and avian tissues by Vero cell culture, TaqMan assay, and RT-PCR assay

Result	No. of samples					
	Mosquito pools			Avian tissues		
	Vero cell culture	TaqMan assay	RT-PCR assay	Vero cell culture	TaqMan assay	RT-PCR assay
Positive	15	15	14	98	96	91
Negative	150	150	151	100	102	107

The TaqMan assay demonstrated a high degree of specific-

TABLE 4. Detection of WN virus in human specimens by TaqMan and RT-PCR assays

Result	No. of samples								
	Brain tissue			CSF			Serum		
	WN virus serology[a]	TaqMan assay	RT-PCR assay	WN virus serology	TaqMan assay	RT-PCR assay	WN virus serology	TaqMan assay	RT-PCR assay
Positive	6	6	5	28	16	0	28	4	0
Negative	28	28	29	30	42	58	19	43	ND[b]

[a] WN virus serology positive is defined as IgM and PRNT assay positivity.
[b] ND, not determined.

ity; no false-positive results were obtained with any of the serologically related flaviviruses tested or with any of the other domestic arthropod-borne viruses tested (Table 2). In contrast, the traditional RT-PCR assay produced a DNA band of the correct size with JE virus (Table 2). Of interest was the observation that the ENV TaqMan primer-probe failed to amplify three of the WN virus strains tested (strains Romania 1996-H, Egypt 1951, and Kunjin 1960). A previous report places the WN virus strains that were positive with the ENV primer-probe (strains NY99, Romania 1996-M, Italy 1998, and Kenya 1998) on the same clade of the phylogenetic tree. These data suggest that the ENV primer-probe is specific for WN virus strains that are closely related to the NY99 strain. The TaqMan 3′NC primer-probe successfully amplified and detected all of the WN virus isolates tested.

The TaqMan assay was able to accurately identify WN virus in field-collected mosquito pools or avian tissues with a degree of sensitivity approaching that of virus isolation in Vero cells. All 15 of the WN virus cell culture-positive mosquito pools were also positive by the TaqMan assay. The single cell culture-positive mosquito pool that was positive for WN virus by the TaqMan assay and negative by the RT-PCR assay produced two plaques in the plaque assay isolation system. Similarly, the two avian tissues that were WN virus isolation positive, TaqMan assay negative, and RT-PCR assay negative produced less than five plaques.

With respect to testing of specimens from humans, the TaqMan and the RT-PCR assays were able to detect WN virus in all (TaqMan assay) or most (RT-PCR assay [five of six specimens]) of the brain tissue specimens obtained at autopsy from patients with WN virus infection. TaqMan and RT-PCR testing of CSF or serum from patients with serologically confirmed cases of WN virus infection was much less successful. Nonetheless, the TaqMan assay detected WN virus in some of the CSF (16 of 28) and serum (4 of 28) samples, whereas none of these samples were positive by the RT-PCR assay. This reduced ability to detect WN virus in CSF or serum from patients with serologically confirmed cases of WN virus infection is likely due to the short-lived viremia in humans with WN virus infection, as has been reported previously (15). As stated above, the use in the traditional RT-PCR assay of alternate primer pairs that amplify a 100-bp region did not alter the results: no positive results for WN virus were obtained by the RT-PCR assay (data not shown). As expected, most of the TaqMan assay-positive CSF specimens were obtained from CSF samples acquired at or before day 12 after the onset of clinical illness, and the viral titers were all less than 10 PFU equivalents, as determined by the quantitative TaqMan assay (Fig. 2). Interestingly, however, WN virus was detected in two patients as late as days 30 and 34; in both of these patients the outcome of infection was fatal.

The recent introduction of WN virus into the northeastern United States necessitates the development of rapid and accurate surveillance for WN virus throughout the Western Hemisphere. The association of WN virus with migratory birds indicates that surveillance for the virus should become a priority, particularly in areas adjacent to migratory bird flyways, including regions of South America. Rapid detection of the virus in

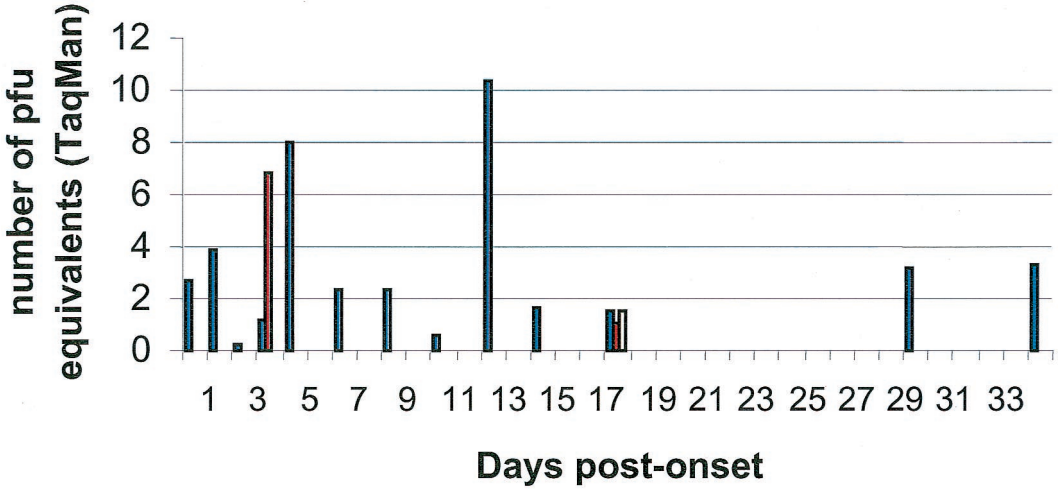

FIG. 2. Estimated quantity of WN virus detected in CSF samples from patients serologically confirmed to have WN virus encephalitis. The viral titers were calculated by generating a standard curve (correlation coefficient, 0.996) with a previously titrated WN virus seed and using the PE 7700 Sequence Detection System software. The results for day 3 represent data from two different specimens, and the results for day 17 represent data for three specimens.

field-collected specimens can accelerate appropriate mosquito control measures that could prevent transmission and disease among humans. The ability to rapidly detect WN virus in clinical human specimens is also significant, given the nonspecificity of the IgM ELISA and the time required to serologically confirm WN virus infection by the PRNT assay. The difficulty in isolating WN virus from human specimens in tissue culture also necessitates the need for a reliable virus detection assay. The TaqMan assay is ideally suited to fulfill these needs since large numbers of samples can be accurately tested in a relatively short time. The data reported from the present study, in hich a large number of samples (≈500) from a variety of sources were tested, indicate that the assay is highly sensitive and specific and could be used along with virus isolation for a comprehensive WN virus detection system in the diagnostic laboratory.

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