Diagnosis of Acute Deer Tick Virus Encephalitis

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Background. Deer tick virus (DTV) is a tick-borne flavivirus that has only recently been appreciated as a cause of viral encephalitis. We describe the clinical presentation of a patient who had DTV encephalitis diagnosed before death and survived for 8 months despite severe neurologic dysfunction.

Methods. Diagnosis was made from a cerebrospinal fluid specimen, using a flavivirus-specific polymerase chain-reaction assay followed by sequence confirmation, and the phylogeny was analyzed. Serologic testing, including plaque reduction neutralization testing, was also performed.

Results. Molecular analysis indicated that the virus was closely related to 2 strains of DTV that had been detected in Ixodes scapularis ticks from Massachusetts in 1996 and in the brain of a patient from New York in 2007.

Conclusions. DTV encephalitis should be considered in the differential diagnosis of encephalitis in geographic areas that are endemic for Lyme disease.

Keywords. deer tick virus; Powassan virus; meningoencephalitis; flavivirus; Ixodes scapularis.

Deer tick virus (DTV) belongs to the group of tick-borne encephalitis RNA viruses in the flavivirus family. Among these viruses, DTV is most closely related genetically to Powassan virus and is sometimes referred to as Powassan virus lineage 2 or, in some publications, Powassan virus lineage 1 [1–3]. Because of this confusing terminology, in this article the prototype strain of Powassan virus will be referred to as POWV and the deer tick virus as DTV. Although DTV and POWV are antigenically related, they seem to be maintained in separate enzootic cycles. DTV is transmitted by the Ixodes scapularis tick. Although this tick species has been shown to be a competent vector for POWV in laboratory studies [4], field-collected I. scapularis ticks have not been found to be infected with POWV, and thus this tick species does not seem to serve as a vector for POWV. To date there are only 2 published cases of DTV encephalitis [5–7]. The first case was reported in 1997 from Ontario, Canada, but was considered to be caused by POWV until genetic sequencing was done many years later [2, 6]. This patient survived and was discharged from the hospital but died of a massive pulmonary embolism 5 weeks after onset of illness. The second patient developed DTV encephalitis in New York State in 2007 [7] and died 17 days after the onset of symptoms while still hospitalized. Both DTV and POWV are causes of a rarely recognized, severe tick-borne meningoencephalitis. In this report, we present the first case of DTV encephalitis diagnosed during the acute illness. The patient was treated with interferon gamma and ribavirin and survived for 8 months but with severe neurologic sequelae.

Clinical Case

A 77-year-old man from Putnam County, New York, presented on 5 December 2010 with fever, dysuria, and lethargy that had begun on 30 November of that year. He had been in Sarasota, Florida, from 29 October to 27 November 2010. Detailed exposure history revealed an active man who frequently gardened. His daughter reported removing an engorged tick attached to his abdomen on 29 October,
immediately before his travel to Florida. His medical history included hypertension, well-controlled type 2 diabetes, hyperlipidemia, chronic obstructive pulmonary disease, and recurrent prostatitis.

At physical examination, the patient had a temperature of 38.3°C, a blood pressure of 101/42 mm Hg, a pulse rate of 106 beats per minute, and a respiratory rate of 20 breaths per minute. His prostate was enlarged and mildly tender. The patient was confused but did not have neck stiffness or focal neurologic signs.

Laboratory results showed a white blood cell count of 11 000 cells/mm³, a hemoglobin level of 14 g/dL, and a platelet count of 142 000/mm³. Renal and liver function test results were within the normal range. *Escherichia coli* (50 000 colony-forming units/mL) grew in a urine culture; blood cultures were negative. The patient was treated with cefepime. On hospital day 4 the patient became comatose, requiring intubation and transfer to the intensive care unit; on the same day he developed myoclonic jerking of his right upper extremity with evidence of neck stiffness. A computed tomographic scan of the head was within normal limits. Cerebrospinal fluid (CSF) showed 256 white blood cells/mm³ with 95% lymphocytes, a glucose level of 140 mg/dL, and a protein level of 133 mg/dL (Table 1). Vancomycin, ampicillin, acyclovir, and fluconazole were added to the patient’s treatment.

A 24-hour electroencephalogram showed episodic epileptic activity in the left frontal central area. On day 8, magnetic resonance imaging of the brain showed enhancement within both cerebellar hemispheres, as well as within the peduncles, pons, and right parahippocampus, consistent with rhombencephalitis (Figure 1). Viral encephalitis screening of the CSF showed the presence of POWV RNA by polymerase chain reaction (PCR). Results of other CSF PCR assays for herpes viruses, arboviruses, adenovirus, and enterovirus were negative. CSF and serum specimens were negative for immunoglobulin G (IgG) and immunoglobulin M (IgM) antibody to West Nile virus (WNV). Lyme disease serologic results were negative. The patient did not improve neurologically despite a 3-week course of pegylated interferon (180 μg/week given subcutaneously) and ribavirin (600 mg twice daily) that was started on hospital day 32. The patient was transferred on day 71 to a chronic care facility. The patient remained comatose and died of pneumonia and respiratory failure 8 months after presentation.

### METHODS

#### Serologic Testing

Serology was performed at the Wadsworth Center at the New York State Department of Health. Microsphere immunoassay (MIA) to detect antibodies (IgA + IgM) to recombinant DTV envelope protein was performed on serum specimens collected on 31 December 2010 and 22 January 2011 [8, 9]. IgM MIA for DTV envelope protein was performed on a CSF specimen collected on 14 December 2010 and on serum specimens collected on 31 December 2010 and 22 January 2011 after IgG depletion and detection by R-phycoerythrin–conjugated anti-IgM [9]. Results for total antibodies and for IgM antibodies were evaluated as a ratio of the median fluorescence intensity (MFI) for 100 beads reacted with the patient’s serum to the MFI of beads reacted with a negative serum specimen.

| Table 1. Oligo Sequences Used in Powassan Molecular Assays |
|-----------------|-----------------|-----------------|
| **Oligo Name**   | **Sequence**     | **Reference**    |
| Real-time primers and probe (POWV) | | |
| Pov9466-F1       | ACCATAACAAACATGAAAGTCCAACT | Previously unpublished |
| Pov9466-F2       | CCATCACAAACATGAAAGTCCAACT | |
| Pov9537-R1       | TGAGTCTGCTGTCCTGATGAC | |
| Pov9537-R2       | CGTGAGTCAGCTGTCCTATGAC | |
| Pov9493         | 6FAM-CTTCCATCATCGGAT-MGB | |
| Conventional PCR and sequencing primers (flavivirus) | | |
| CFD2             | GTGTCCCAACGGCGCGTTGTCATCAGC | Scaramozzino et al [11] |
| MAMD             | AACATGATGGGRAARAGRGARAA | |
| FS778            | AARGGHAYMCDGCHATHTGGT | |
| Conventional PCR and sequencing primers (DTV) | | |
| DT-F3            | AGCCGTTCCTCCTGATGTTGA | Tavakoli et al [7] |
| DT-10105R        | CCATGTCAGCGTTGTCGATAA | |
| DT-9178          | GGTTGGTGCTGTCAGGAGCT | |
| DT-9695-F        | GTAAGGATGCTGCGGAGT | |
| DT-R3            | CATGAAATGGTGAGCAG | Previously unpublished |

Abbreviations: DTV, deer tick virus; PCR, polymerase chain reaction; POWV, Powassan virus.
The cutoff for a positive result was a value of 3 standard deviations above the mean MFI result based on a panel of serum specimens from healthy subjects. The positive control serum was from a patient from Dutchess County, New York, who had tested positive for POWV antibodies by cross-species plaque reduction neutralization tests (PRNTs) at the Wadsworth Center. Recombinant DTV envelope protein was produced from the DTV-Ipswich strain, as described elsewhere [8, 9]. Neutralization tests against POWV and DTV were performed at the Wadsworth Center.

**Molecular Analysis**

Samples of CSF collected on 14 December 2010 and 6 January 2011 were tested with molecular assays. Total nucleic acid was extracted from CSF with an easyMAG instrument (bioMérieux), and real-time PCR assays described elsewhere [10] were performed for the detection of viruses causing encephalitis. These assays targeted herpes simplex virus types 1 and 2, varicella-zoster virus, cytomegalovirus, Epstein-Barr virus, adenovirus, human herpesvirus 6, enterovirus, WNV, Eastern equine encephalitis virus, and St Louis encephalitis virus (SLEV). A 1-step, real-time reverse-transcriptase (RT) PCR assay for the detection of POWV and DTV was also performed.

Two conventional heminested RT-PCR assays were used to generate material for sequencing, both targeting the nonstructural protein 5 (NS5) region of DTV. One assay [11] amplified a 220-base pair (bp) fragment, and the other [7] amplified an overlapping 873-bp fragment. Technical details of both PCR assays and the dideoxy sequencing are provided below. Edited combined sequences provided a 981-bp region of the viral genome for phylogenetic analysis. (Additional molecular studies were approved under New York State Department of Health Institutional Review Board study number 02-054.)

Figure 1. Magnetic resonance images of the brain with gadolinium enhancement on day 8 of hospitalization (top row) compared with day 51 (bottom row). Images demonstrate enhancement within the bilateral cerebellar hemispheres on day 8 (A), which was markedly improved on day 51 (B); however, new enhancement was seen on day 51 in the right basal ganglia (C), which had been absent at presentation (D).
Real-time PCR
A 1-step real-time RT-PCR assay that detects both lineages of Powassan virus (DTV and POWV) was performed using the Invitrogen SuperScript III Platinum One-Step qRT-PCR System. Each 25-µL reaction comprised 6.05 µL of RNase-free water, 12.5 µL of 2x reaction mix, 0.35 µL of each of the 2 forward primers and the 2 reverse primers at 25 µmol/L, 0.15 µL of the probe at 25 µmol/L, 0.25 µL of SuperScript III Platinum Taq mix, and 5 µL of extracted nucleic acid as template. One-step thermal cycling conditions were as follows: an initial RT step for 30 minutes at 50°C, followed by a 15-minute Taq enzyme activation step at 95°C, then 45 cycles of 95°C for 15 seconds and 60°C for 1 minute. The thermal cycling conditions were as follows: an initial RT step for 30 minutes at 50°C, followed by a 15-minute activation step at 95°C, then 45 cycles of 95°C for 15 seconds and 60°C for 1 minute.

Small-Fragment Conventional RT-PCR
The conventional heminested primer sets [11] produce a 250-bp amplicon in the first round and a 220-bp amplicon in the second round. The assay targets a conserved region of the NS5 gene using primer sequences shown in Table 1. Reaction conditions, modified from the original publication, are listed below. The first-round RT-PCR reaction used the OneStep RT-PCR kit (Qiagen) in a 50-µL total volume consisting of 29 µL of RNase-free water, 10 µL of 5x OneStep RT-PCR Buffer (12.5 mmol/L magnesium chloride), 2 µL of 10 mmol/L dNTP mix, 1 µL of each first-round primer at 25 µmol/L, 2 µL of OneStep RT-PCR Enzyme Mix, and 5 µL of extracted sample RNA. Thermal cycling conditions were as follows: an initial RT step for 30 minutes at 50°C, followed by a 15-minute Taq enzyme activation step at 95°C, then 35 cycles of 94°C, 53°C, and 72°C, each for 1 minute, with a final 72°C extension step of 10 minutes. The subsequent 50-µL heminested PCR reaction comprised 36.75 µL of RNase-free water, 5 µL of 10× Qiagen PCR buffer (1.5 mmol/L magnesium), 1 µL of 10 mmol/L dNTP, 0.25 µL of Qiagen HotStar Taq enzyme (5 units/µL), and 3 µL of each primer at 10 µmol/L, with 5 µL of first-round PCR product as template. The thermal cycling conditions were as follows: an initial activation step of 95°C for 15 minutes, followed by 45 cycles of 95°C for 30 seconds, 56°C for 1 minute, and 72°C for 75 seconds, with a final 10-minute extension step at 72°C.

Sequence Analysis
All PCR products were analyzed on a 2% TBE agarose gel stained with SYBR Safe DNA gel stain (Invitrogen) and were purified using the Zymoclean Gel DNA Recovery kit (Zymo Research) or PCR purified using Quantum Prep PCR Kleen Spin Columns (Bio-Rad). The Wadsworth Center Applied Genomic Technologies Core performed sequencing on an ABI 3730 instrument, using either the nested PCR primers CFD2 and FS778 or primers DT-9178, DT-10105R, DT-9695-F, and DT-R3 (Table 1).

RESULTS
Immunology
The MIA testing on CSF collected on hospital day 9 was IgM reactive to DTV envelope protein, with a patient–to–negative control ratio of 36.8. Blood collected on days 26 and 48 showed a strongly positive serum IgM and polyvalent (IgG + IgA + IgM) MIA to DTV envelope protein. The PRNT titer was elevated at 5120 for POWV-specific neutralizing antibodies, whereas the PRNT titer for a DTV strain, isolated from I. scapularis ticks collected in Westchester County in 2008, was also elevated, but at 80. This paradoxical phenomenon remains to be elucidated but has been noted before [2, 12] and might be related to the particular viral strains used in the testing.

An enzyme-linked immunosorbent assay (ELISA) of blood collected on hospital day 48 was tested at the Centers for Disease Control and Prevention (CDC) and was found to be positive for IgM and separately for IgG antibodies to POWV envelope protein, with a PRNT titer of 20 480. CSF collected on hospital day 48 was tested at the Centers for Disease Control and Prevention (CDC) and was found to be positive for IgM and separately for IgG antibodies to POWV envelope protein, with a PRNT titer of 20 480.
antibodies by ELISA, with PRNT titer of 8192 when tested at the CDC (Table 2).

**Molecular Analysis**

POWV or DTV was detected by real-time RT-PCR in the CSF sample collected on 14 December 2010 but not in the CSF specimen from 6 January 2011. Amplification signal strength (cycle threshold, 35.4) indicated that the viral load on 14 December 2010 was very low. Results of real-time PCR assays for all other viruses were negative. Conventional RT-PCR assays for DTV, performed on the CSF specimen from 14 December 2010, produced amplicons for sequencing, which were edited and assembled for phylogenetic assessment. Analysis indicated that the virus was closely related to 2 strains of DTV that had been detected in *I. scapularis* ticks from Massachusetts in 1996 and from a prior clinical case from New York in 2007 (Figure 2).

**DISCUSSION**

This is the third case of DTV encephalitis described in detail in the published literature. Although the patient was treated with ribavirin plus interferon, it is unclear whether this therapy played any beneficial role. A notable aspect of our case was the lengthy incubation period from the known tick bite 32 days before onset of symptoms. Data from cases of POWV infection indicate an incubation period ranging from 7 to 34 days [13–15]. Based on the time of year when the tick bite occurred (29 October 2010) and the geographic location of the patient in the Lower Hudson Valley of New York State, this tick was most likely an adult *I. scapularis*. DTV was first isolated in 1997 from *I. scapularis* ticks collected in coastal New England [16]. Although the first recognized human case of DTV encephalitis also occurred in 1997 [6], proof based on sequence data was not available until 2001 [2]. The DTV genome has been completely sequenced (10.8...
Kb). It shares 84% nucleotide sequence and 94% amino acid sequence identity with POWV [2]. The 2 viruses share a common ancestral origin in North America, from which they diverged into 2 different lineages about 485 years ago and have adapted to different vector hosts with overlapping geographic distribution in Canada and United States [3]. Both viruses are regarded as antigenically indistinguishable (despite the markedly different PRNT titers observed in this patient); therefore, genotypic analysis is needed for definitive diagnosis.

Serologic assays for DTV and POWV can also cross-react with WNV, SLEV, and other flaviviruses; therefore, immunofluorescent assays need to be confirmed either with specific neutralizing antibody assays or by PCR [12].

POWV is found in *Ixodes cookei*, *Ixodes marxi*, and *Ixodes spinipalpus* but thus far not in *I. scapularis*. DTV which was originally isolated from *Dermacentor andersoni* ticks is mainly found in *I. scapularis* [17]. The principal reservoir hosts for POWV are the groundhog (*Marmota monax*) and the striped skunk (*Mephitis mephitis*) [18], whereas DTV is maintained in a separate enzootic cycle between *I. scapularis* and, presumably, the white-footed mouse (*Peromyscus leucopus*) [16]. Based on PCR data and genomic sequencing, infection rates with DTV in the range of 0.43%–5.0% have been found in adult *I. scapularis* ticks in several geographic areas including Nantucket Island (Massachusetts), Prudence Island (Rhode Island), Bridgeport and northwestern Connecticut [16, 19, 20], the cities of Spooner and Hayward in Wisconsin [21, 22], Suffolk County in Long Island, New York, and Westchester County, which is in the Lower Hudson Valley of New York State [23]. Between 3% and 4% of mice from both Spooner, Wisconsin, and Nantucket Island were found to have serologic evidence of exposure to POWV or DTV [19].

![Figure 2. Evolutionary relationship of 18 taxa inferred using the neighbor-joining method [28] with bootstrap (500 replicates) [29]. Relatedness was supported by analysis with the maximum parsimony method from a total of 981 positions in the final data set. Branches corresponding to partitions reproduced in <50% of bootstrap replicates are collapsed. Evolutionary distances were computed using the data-specific method [30], and units represent the number of base substitutions per site. Evolutionary analyses were conducted using MEGA4 software [31]. RS9266 [2], DT-NY-07 [7], and DTV-MN-2008 (not reported) are the only known DTV strains from human cases. The newly identified strain is in bold type and underlined. DTV, deer tick virus; POWV, Powassan virus.](https://academic.oup.com/cid/article-abstract/56/4/e40/352454/0.43-5.0% have been found in adult *I. scapularis* ticks in several geographic areas including Nantucket Island (Massachusetts), Prudence Island (Rhode Island), Bridgeport and northwestern Connecticut [16, 19, 20], the cities of Spooner and Hayward in Wisconsin [21, 22], Suffolk County in Long Island, New York, and Westchester County, which is in the Lower Hudson Valley of New York State [23]. Between 3% and 4% of mice from both Spooner, Wisconsin, and Nantucket Island were found to have serologic evidence of exposure to POWV or DTV [19]. Transstadiad
transmission of DTV occurs at a rate of only 22% experimentally [24]. Transovarial transmission of POWV in *I. scapularis* ticks has been documented at a rate of 16.6% [4].

About 70 human cases of Powassan virus encephalitis have been reported as of 2012; most of the cases reported since 2010 were diagnosed in the states of Minnesota and Wisconsin [5,25]. It is important to be aware that the vast majority of the cases reported as POWV were not diagnosed with the aid of genetic sequencing and thus an unknown fraction of them may have actually been due to DTV. Of the 11 cases reported from New York State between 2007 and 2010, 7 were from Westchester and Putnam counties, which are highly endemic for Lyme disease [25]. Although about 2.2% of *I. cookei* ticks were found to be infected with POWV in southern New England [21], humans are much more likely to be bitten by *I. scapularis* ticks than by *I. cookei* or *I. marxi* ticks [5, 26]. It should be noted also that transmission of DTV in mouse systems can occur within just 15 minutes after tick attachment, which is much more rapid than for the other recognized pathogens transmissible by this tick species [24]; thus, it seems plausible that there may be many subclinical infections with DTV.

Infection with POWV/DTV is diagnosed by the presence of any of the following: (1) detection of POWV/DTV RNA in CSF specimens; (2) detection of POWV/DTV-specific IgM in CSF specimens; (3) presence of a ≥4-fold increase in POWV/DTV-specific neutralizing antibodies in serial serum specimens; or (4) detection of POWV/DTV-specific IgM in a serum specimen and POWV/DTV-specific neutralizing antibodies in the same or a later specimen. Neutralizing antibodies are considered POWV/DTV specific if the POWV/DTV titer is ≥4-fold higher than the titers to WNV and SLEV [12]. Testing can be obtained at certain state public health laboratories or at the CDC. DTV can be differentiated from POWV by genomic sequencing using primers from the prototype DTV envelope coding region and the NS5 coding region [27].

In conclusion, DTV encephalitis is a rarely recognized cause of encephalitis in geographic areas endemic for Lyme disease. Although serologic methods are more commonly used for diagnosis of POWV/DTV encephalitis, the initial and definitive diagnosis in our case was made with molecular testing. Moreover, sequence analysis enabled assessment of the phylogenetic relationship between this DTV strain and other POWV and DTV strains reported in the literature and public databases.

**Notes**

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