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別紙様式第2-1

No. 9

医薬品　研究報告　調査報告書

識別番号・報告回数			報告日	第一報入手日	新医薬品等の区分		総合機構処理欄
				2009. 4. 15	該当なし		
一般的名称	人赤血球濃厚液						
販売名（企業名）	赤血球濃厚液-LR「日赤」(日本赤十字社) 照射赤血球濃厚液-LR「日赤」(日本赤十字社)		研究報告の公表状況	Nóbrega AA, Garcia MH, Tatto E, Obara MT, Costa E, Sobel J, Araujo WN. Emerg Infect Dis. 2009 Apr;15(4):653-5.	公表国	ブラジル	

研究報告の概要	○ブラジルにおけるアサイー果実摂取によるシャーガス病の経口伝播 2006年1月～11月にブラジルアマゾンのパラー州で、急性シャーガス病合計178症例が報告され、このうち一部でアサイー果実の摂取による経口伝播の可能性が判明した。 Barcarenaで発症した11例は、血液スメア検体の観察で原虫が確認された。後方視的コホート試験および症例対照試験を実施した。輸血歴、臓器移植歴、森林地帯での滞在、サシガメに刺されたことについては全員が否定した。11名中5名は、9月15日に行われた会合で同じものを食べており、アサイーのペーストやジュースの摂取が共通の暴露要因だった。アサイー果実を潰す際に、原虫を媒介するサシガメの排泄物が混入した可能性が考えられた。
報告企業の意見	ブラジルで発生したシャーガス病のアウトブレイクにおいて、アサイー果実の摂取による経口伝播の可能性が判明したとの報告である。
今後の対応	日本赤十字社は、輸血感染症対策として献血時に海外渡航歴の有無を確認し、帰国(入国)後4週間は献血不適としている。また、シャーガス病の既往がある場合には献血不適としている。日本在住の中南米出身献血者については、厚生労働科学研究「献血血の安全性確保と安定供給のための新興感染症等に対する検査スクリーニング法等の開発と献血制限に関する研究」班と共同して検討する予定である。今後も引き続き情報の収集に努める。
使用上の注意記載状況・その他参考事項等	赤血球濃厚液-LR「日赤」 照射赤血球濃厚液-LR「日赤」 血液を介するウイルス、細菌、原虫等の感染 vCJD等の伝播のリスク

(3)

Oral Transmission of Chagas Disease by Consumption of Açaí Palm Fruit, Brazil

Aglaêr A. Nóbrega, Marcio H. Garcia, Erica Tatto, Marcos T. Obara, Elenild Costa, Jeremy Sobel, and Wildo N. Araujo

In 2006, a total of 178 cases of acute Chagas disease were reported from the Amazonian state of Pará, Brazil. Eleven occurred in Barcarena and were confirmed by visualization of parasites on blood smears. Using cohort and case–control studies, we implicated oral transmission by consumption of açaí palm fruit.

Chagas disease (American trypanosomiasis) chronically infects ≈10 million persons in Latin America (*1*). The etiologic agent is *Trypanosoma cruzi*, which is transmitted by bloodsucking triatomine insects. Other modes of transmission are transfusional, congenital, and oral (foodborne) (*2*). Oral transmission occurs by consumption of foods contaminated with triatomines or their feces or by consumption of raw meat from infected mammalian sylvatic hosts (*3*). The precise stage of food handling at which contamination occurs is unknown. The first outbreak of orally transmitted Chagas disease in Brazil was reported in 1965 (*4*). Two outbreaks were associated with consumption of sugar cane juice (*5,6*). In these outbreaks, the incubation period was ≈22 days, compared with 4–15 days for vectorial transmission and 30–40 days for transfusional transmission (*7*).

Chagas disease has not been considered endemic in the Brazilian Amazon region. The first Amazonian outbreak of acute Chagas disease was reported in 1968; oral transmission was suspected (*8*). During 1968–2005, a total of 437 cases of acute Chagas disease were reported in this region. Of these cases, 311 were related to 62 outbreaks in which the suspected mode of transmission was consumption of açaí (*9*).

Açaí is the fruit of a palm of the family *Aracaceae* (Figure 1, panel A); it is crushed to produce a paste or beverage. Most of the Amazonian population consumes açaí juice daily. Contamination is believed to be caused by triatomine stools on the fruit or insects inadvertently crushed during processing (*10*). There are no reports of collection of açaí for laboratory testing during an outbreak of acute Chagas disease. Because outbreaks with high attack rates occur in small groups whose members all consume the same foods, açaí has not been epidemiologically implicated in transmission of this disease.

During January–November 2006, a total of 178 cases of acute Chagas disease were reported in Pará State, Brazil, in the Amazon basin (Ministry of Health, unpub. data). Eleven of these cases occurred in Barcarena (population 63,268) (*11*) (Figure 1, panel B). All patients had symptom onset in September and October. Of the 11 case-patients, 5 were staff members at a health post who shared a meal at a staff meeting on September 15. We attempted to identify risk factors for illness.

The Study

We conducted a retrospective cohort study of staff members at the health post who participated in the meeting on September 15. A case-patient was any person who participated in the meeting and had a positive direct parasitologic examination for *T. cruzi* or positive serologic results and clinical evidence of acute Chagas disease. A non-case was any person who participated in the meeting and had negative test results for *T. cruzi*. We also conducted a 1:3 case–control study (11 case-patients and 34 controls matched by sex and age) that included patients with laboratory confirmed cases from Barcarena. A case-patient was any person in whom during September 1–October 15 *T. cruzi* was found by direct parasitologic examination, irrespective of signs or symptoms of disease, or who had positive serologic results and clinical evidence of disease. This interval was based on date of symptom onset of the first and last case-patient and a reported incubation period of 3–22 days for orally transmitted disease. Controls were age- and sex-matched residents of case-patient neighborhoods who had negative serologic results for *T. cruzi*.

Parasitologic examinations were conducted for case-patients by using quantitative buffy coat test, thick blood smear, or buffy coat test (the latter 2 tests included Giemsa staining). Serologic tests were conducted by using indirect hemagglutination test, ELISA, or indirect immunofluorescent test. An immunoglobulin (Ig) M titer ≥40 was considered positive. Controls had nonreactive IgM and IgG titers. We ruled out leishmaniasis in all persons with positive serologic results for *T. cruzi* by using an immunofluorescent test for IgM to *Leishmania* spp. (*12*).

We conducted an entomologic investigation during December 11–16, 2006, at the homes of 5 case-patients and in forested areas near the homes of 2 case-patients; at the commercial establishment where açaí consumed by the case-patients linked to the health post was prepared and served; at an açaí juice production and sale establishment reported to be frequented by other case-patients; and at the river dock market where açaí delivered to Barcarena is unloaded. At this market, we searched baskets used to transport açaí in river boats. We applied an insect-displacing compound (piridine; Pirisa, Taquara, Brazil) to the interior and exterior of buildings at investigation sites and placed traps (*13*) to obtain triatomines.

Data were analyzed by using Epi Info version 6.04d (Centers for Disease Control and Prevention, Atlanta, GA, USA). We measured relative risk in the cohort study and matched odds ratios in the matched case–control study, with 95% confidence intervals and $\alpha = 5\%$. Fisher exact, McNemar, Mantel-Haenszel, and Kruskall-Wallis tests were used as needed. Study power $(1 - \beta)$ was 5%.

All case-patients had positive results for *T. cruzi* by direct examination of blood (Figure 2). Nine (82%) patients were female; median age was 39 years (range 7–70 years). Eight (73%) patients resided in urban areas, 7 (64%) in brick dwellings, and 3 (27%) in mixed brick and wooden dwellings. All patients denied having had blood transfusions or organ transplants, having slept in rural or sylvatic areas, and having been bitten by triatomines.

The epidemic curve for the 11 patients is shown in Figure 1, panel C. Main signs and symptoms were fever, weakness, facial edema, myalgia, arthralgia, and peripheral edema (Table 1). No deaths occurred, and median time from symptom onset to treatment initiation was 22 days.

The cohort consisted of 12 persons who attended the staff meeting. Of these persons, 6 shared a meal, 5 (83%) of whom were case-patients. The remaining persons were seronegative for *T. cruzi*. Exposures associated with infection were consumption of thick açaí paste and drinking açaí juice at the health post; consumption of chilled açaí was protective (Table 2). This shared meal was the only common exposure among cohort members. No other foods consumed at the meal were associated with illness (Table 2). Among exposures tested, drinking açaí juice on September 15 and at the health post were significantly associated with illness ($p<0.02$ and $p<0.001$, respectively; matched odds ratio not determined). Other exposures were not associated with illness. No triatomine insects were identified at any sites of the entomologic investigation.

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DOI: 10.3201/eid1504.081450

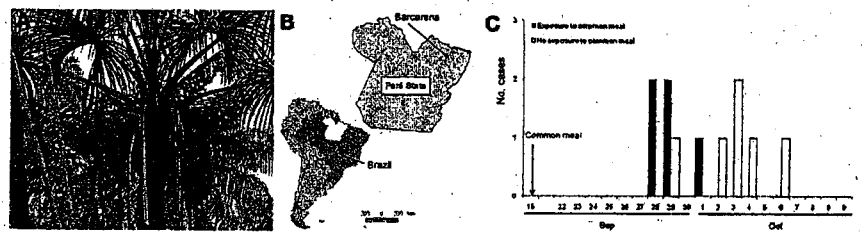

Figure 1. A) Açaí palm and açaí fruit. B) Location of Barcarena in Pará State, Brazil. C) Epidemic curve for 11 case-patients with acute Chagas disease, Barcarena, Brazil, September–October 2006.

Figure 2. *Trypanosoma cruzi* (arrow) in a peripheral blood smear of a patient at a local health facility in a rural area of Pará State, Brazil (Giemsa stain, magnification ×100). Image provided by Adriana A. Oliveira, Brazilian Field Epidemiology Training Program, Brasília, Brazil.

Table 1. Signs and symptoms in 11 patients with laboratory-confirmed acute Chagas disease, Barcarena, Brazil, 2006

Sign or symptom	No. (%) patients
Fever	11 (100)
Fatigue	11 (100)
Facial edema	11 (100)
Headache	10 (91)
Myalgia	9 (82)
Arthralgia	9 (82)
Peripheral edema	9 (82)
Shortness of breath	7 (64)
Tachycardia	7 (64)
Nausea/vomiting	7 (64)
Jaundice	5 (46)
Epigastric pain	5 (46)
Retroorbital pain	5 (46)

Table 2. Food exposures in a cohort study of 5 case-patients with acute Chagas disease, Barcarena, Brazil, 2006*

Exposure†	Ill, no. (%)	Not ill, no. (%)	RR	95% CI	p value‡
Açaí, thick paste	3 (100)	0	4.5	1.3–15.3	0.04
Açaí juice at health post	3 (100)	0	4.5	1.3–15.3	0.04
Chilled açaí juice	1 (12)	7 (88)	0.1	0.02–0.8	0.02
Charque	3 (75)	2 (25)	5.3	0.8–35.1	0.09
Cupuaçu	2 (100)	0	3.3	1.3–8.6	0.15
Biribá	1 (50)	1 (50)	1.3	0.3–6.1	0.68
Muruci	1 (100)	0	2.3	1.3–6.0	0.42
Any raw food	4 (67)	2 (33)	4.0	0.6–26.1	0.12

*RR, relative risk; CI, confidence interval.
†Charque is dried, salted meat; cupuaçu, biribá, and muruci are fruits.
‡By Fisher exact test.

Conclusions

Our study findings implicated açaí in an outbreak of acute Chagas disease. Oral transmission of this disease in the Amazon region has been reported since the 1960s. Açaí has long been the principal suspected food vehicle, but characteristics of outbreaks, small groups with universal exposure and high attack rates, have precluded epidemiologic implication of this food. There are no reports of timely collection of açaí for laboratory testing in an outbreak.

In this outbreak, vectorborne, transfusional, transplant-associated, and transplacental transmission were excluded. Incubation periods of cohort case-patients were compatible with those of previous reports. A shared meal was the only event linking case-patients, and cohort and case–control studies demonstrated an association between açaí consumption at this meal and infection. These findings indicate an outbreak of orally transmitted disease from contaminated açaí.

Limitations of this study are possible recall bias caused by delay between illness and investigation and failure to collect food samples for testing. Studies are needed to determine viability of *T. cruzi* in açaí, along with the tree-to-bowl continuum of açaí, to identify sources of contamination. Because açaí is a major dietary component in the Amazon region and a component of the local economy, identifying practical prevention measures is essential.

Ms Nóbrega is supervisor of the Field Epidemiology Training Program of the Brazilian Ministry of Health in Brasília, Brazil. Her research interests include the epidemiology of infectious diseases and outbreak investigations.

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医薬品 研究報告 調査報告書

識別番号・報告回数			報告日	第一報入手日	新医薬品等の区分		総合機構処理欄	
				2009. 4. 9	該当なし			
一般的名称	人赤血球濃厚液		研究報告の公表状況	ProMED 20090406.1328, 2009 Apr 6. 情報源: El Universal, 2009 Apr 5.	公表国	ベネズエラ	使用上の注意記載状況・その他参考事項等	
販売名（企業名）	赤血球濃厚液-LR「日赤」(日本赤十字社) 照射赤血球濃厚液-LR「日赤」(日本赤十字社)						赤血球濃厚液-LR「日赤」 照射赤血球濃厚液-LR「日赤」	
研究報告の概要	○食品介在性トリパノソーマ症 - ベネズエラ、グアバジュース ベネズエラ北部のバルガス州西部Chichiriviche de la Costaの住民らに被害が出ている疾患は、シャーガス病であることが確認された。汚染されたグアバジュースの摂取により伝播され、同じ学校に通う児童47名と教師3名が感染するアウトブレイクが発生した。4週間以上続く流行で患者数は増加しており、7、9、12歳の3名の児童が死亡した。児童35名は未だ入院中で、重症患者もいる。既に対策が取られ、感染拡大の危険はない。						血液を介するウイルス、細菌、原虫等の感染 vCJD等の伝播のリスク	
	報告企業の意見		今後の対応					
	ベネズエラで、グアバジュースの摂取によるシャーガス病のアウトブレイクが発生し、同じ学校に通う児童47名と教師3名が感染、児童3名が死亡したとの報告である。		日本赤十字社は、輸血感染症対策として献血時に海外渡航歴の有無を確認し、帰国（入国）後4週間は献血不適としている。また、シャーガス病の既往がある場合には献血不適としている。日本在住の中南米出身献血者については、厚生労働科学研究「献血血の安全性確保と安定供給のための新興感染症等に対する検査スクリーニング法等の開発と献血制限に関する研究」班と共同して検討する予定である。今後も引き続き情報の収集に努める。					

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Archive Number 20090406.1328
Published Date 06-APR-2009
Subject PRO/AH/EDR> Trypanosomiasis, foodborne - Venezuela: (Vargas), guava juice

TRYPANOSOMIASIS, FOODBORNE - VENEZUELA: (VARGAS), GUAVA JUICE
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A ProMED-mail post
<http://www.promedmail.org>
ProMED-mail is a program of the
International Society for Infectious Diseases
<http://www.isid.org>

Date: 5 Apr 2009
Source: El Universal [trans by Mod.MPP, edited]
<http://www.eluniversal.com/2009/04/05/grccs_art_confirman-chagas-en_1338174.s

Chagas confirmed on the west coast of Vargas

Ministry of Health [MINSA] reiterates the lifting of epidemiologic siege

Yesterday the Minister of Health, Jesus Mantilla, confirmed that Chagas disease is the disease that is attacking the population of Chichiriviche de la Costa, in the western part of the state of Vargas.

The head of the Ministry of Health was in the area and stated that it was transmitted through the ingestion of contaminated guava juice, producing the outbreak of illness in the area, that affected 47 students and three teachers from the morning shift of the Romulo Monasterios state school.

Similarly, the minister reiterated the statements made yesterday [4 Apr 2009 -- see prior ProMED-mail posting Undiagnosed fatalities - Venezuela (02): (Vargas) Chagas susp, RFI 20090404.1305 - Mod.MPP] by the governor of Vargas, Jorge Garcia Carneiro, the epidemiologic "fence" erected to stop the epidemic that occurred in the area, because, as noted, there is no risk of spread.

For this disease, which for over 4 weeks was affecting the population and increasing numbers of patients, killing 3 children ages 7, 9 and 12 years.

However, 35 other children remain hospitalized in the La Guaira Social Security [hospital], the Pariata Periferico [health facility], the Perez Carreno [health facility] and the University Clinic. Doctors from this hospital reported that 15 patients from the area have been admitted, and that the problem is present from [the events surrounding carnaval - Mardis Gras - Mod.MPP]. It was learned that there is a patient in serious condition.

Although the possibility of transmission in the zone was ruled out, the residents of Chichiriviche reported that the usual vacationers to the zone have not arrived. [The affected area is a beach resort frequented by vacationers. The week ending in Easter Sunday is known as Semana Santa in Latin American countries. It is a vacation week, and locations such as Chichiriviche are usually filled with vacationers coming for the week. - Mod.MPP]

[Byline: Anthony Rangel]

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Communicated by:
ProMED-mail <promed@promedmail.org>

[The above newswire is confirmation of the suspicion that the previously undiagnosed outbreak in Venezuela (see prior ProMED-mail postings listed below) is due to ingestion of a juice that was contaminated with _Triatoma infestans_ intestinal contents.

This is now the 7th outbreak of foodborne transmission of trypanosomiasis in the Americas reported by ProMED-mail (see prior postings listed below). As mentioned in the 1st report of this current outbreak (Undiagnosed fatalities - Venezuela: (Vargas), Chagas, susp, RFI 20090402.1279), the 1st reported outbreak of foodborne transmission of trypanosomiasis was reported in Santa Catarina Brazil in 2005 (see prior ProMED-mail postings listed below). This outbreak was associated with ingestion of sugar cane juice that was found to be contaminated with crushed _Triatoma infestans_, the vector of trypanosomiasis in Brazil. Since reporting of outbreaks of foodborne transmitted trypanosomiasis began, there were 6 prior documented outbreaks associated with contaminated juices -- 4 in Brazil (involving 4 states in the country), one in Venezuela, and one in Colombia. The prior outbreak in Venezuela involved 128 cases at a school in metropolitan Caracas, and was associated with contaminated fruit juice. This current outbreak has involved approximately 50 cases at a school in a small beachside town/village outside of Caracas, and is also associated with contaminated fruit juice.

One wonders how new a phenomenon foodborne transmission of trypanosomiasis really is, or is it just that we are now looking more carefully as the standard of housing in these countries has improved, and exposure to the _Triatoma infestans_ in the household has decreased. Or perhaps, there is improved recognition and investigation of acute outbreaks in general in the region.

For the interactive HealthMap/ProMED map of Chichiriviche with links to other recent ProMED-mail postings in surrounding areas, see <http://healthmap.org/r/008y>. - Mod.MPP]

[see also:
Undiagnosed fatalities - Venezuela (02): (Vargas) Chagas susp, RFI 20090404.1305
Undiagnosed fatalities - Venezuela: (Vargas), Chagas, susp, RFI 20090402.127
Trypanosomiasis - Colombia: (SAN), foodborne susp. 20090121.0259
2007

Trypanosomiasis, foodborne - Venezuela: (Caracas) (02) 20071231.4192
Trypanosomiasis, foodborne - Venezuela: (Caracas) 20071226.4141
Trypanosomiasis, foodborne - Brazil (Amazonia) 20070821.2732
2006

Trypanosomiasis, foodborne - Brazil (PA) 20060728.2085
2005

Trypanosomiasis, foodborne - Brazil (Santa Catarina) (05) 20050401.0940
Trypanosomiasis - Brazil (Amapa) 20050331.0929
Trypanosomiasis, foodborne - Brazil (Santa Catarina) (04) 20050330.0917
Trypanosomiasis, foodborne - Brazil (Santa Catarina) (03) 20050327.0884
Trypanosomiasis, foodborne - Brazil (Santa Catarina) (02) 20050325.0870
Trypanosomiasis, foodborne - Brazil (Santa Catarina) 20050324.0847
1997

Chagas disease - Latin America 19970114.0066
Chagas disease vector (05) 19970118.0105
1996

Trypanosomes, New World, Symposium - Guyana 1996 19960830.1493]
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研究報告の概要

New York の 62 才男性は，シカダニウイルスに感染したシカダニの咬傷後，髄膜脳炎で死亡した。手術および剖検で採取された組織標本の解析で、広範囲にわたる壊死性髄膜脳炎であることが明らかになった。ホルマリン固定組織から核酸が抽出され、シカダニウイルスの存在がフラビウイルス特異的 PCR 測定法で確認された。

シカダニウイルスは、フラビウイルスのダニ媒介脳炎群であり、ポワッサンウイルスと密接に関係がある。ダニ媒介脳炎ウイルスとポワッサンウイルスを含めて、フラビウイルスのダニ媒介脳炎群のいくつかは、人および動物で脳炎を起こす。ダニ媒介脳炎ウイルスは最も重大な大発生を起こしている。これらのウイルスは抗原性において密接に関連し、主に北半球で見つかっている。ダニ媒介脳炎ウイルスによる感染は軽度あるいは無症候性、または、髄膜炎と脳炎が起こる可能性がある。

米北東部および北中央部の一定の地域で、シカのシカダニウイルスの保有率は高い。しかし、ヒト感染は過去に報告されていない。これは、このウイルスが容易に人に感染しない、あるいは、それが特に病原性でないことを示唆する。脳炎症状患者においてポワッサンウイルスの診断検査は通常実施されない。

そのため、ヒト発生率は、過小評価される可能性がある。

シカダニはライム病、ヒト・バベシア症やヒト顆粒球アナプラズマ症を含むいくつかのダニ媒介疾患を伝染させる。この症例は、シカダニウイルスが致命的脳炎の原因でありえることを立証する。

報告企業の意見	今後の対応
シカダニウイルスがヒトに感染した初めての報告であり、また、このウイルスが致命的脳炎の原因であり得とする報告である。 シカダニウイルスは、フラビウイルス科フラビウイルス属に属し、ビリオンは球形で、直径 40～50nm のエンベロープ有する RNA ウイルスである。万一、原料血漿にシカダニウイルスが混入しても、BVD をモデルウイルスとしたウイルスバリデーション試験成績から、製造工程において十分に不活化・除去されると考えている。	本報告は本剤の安全性に影響を与えないと考えるので、特段の措置はとらない。

使用上の注意記載状況・その他参考事項等

2. 重要な基本的注意

(1) 本剤の原材料となる献血者の血液については、HBs 抗原、抗 HCV 抗体、抗 HIV-1 抗体、抗 HIV-2 抗体、抗 HTLV-I 抗体陰性で、かつ ALT(GPT)値でスクリーニングを実施している。更に、プールした試験血漿については、HIV-1、HBV 及び HCV について核酸増幅検査(NAT)を実施し、適合した血漿を本剤の製造に使用しているが、当該 NAT の検出限界以下のウイルスが混入している可能性が常に存在する。本剤は、以上の検査に適合した血漿を原料として、Cohn の低温エタノール分画で得た画分から人ハプトグロビンを濃縮・精製した製剤であり、ウイルス不活化・除去を目的として、製造工程において 60℃、10 時間の液状加熱処理及びウイルス除去膜によるろ過膜処理を施しているが、投与に際しては、次の点に十分注意すること。

BRIEF REPORT

Fatal Case of Deer Tick Virus Encephalitis

Norma P. Tavakoli, Ph.D., Heng Wang, M.A., Michelle Dupuis, B.Sc., Rene Hull, B.A., Gregory D. Ebel, Sc.D., Emily J. Gilmore, M.D., and Phyllis L. Faust, M.D., Ph.D.

SUMMARY

Deer tick virus is related to Powassan virus, a tickborne encephalitis virus. A 62-year-old man presented with a meningoencephalitis syndrome and eventually died. Analyses of tissue samples obtained during surgery and at autopsy revealed a widespread necrotizing meningoencephalitis. Nucleic acid was extracted from formalin-fixed tissue, and the presence of deer tick virus was verified on a flavivirus-specific polymerase-chain-reaction (PCR) assay, followed by sequence confirmation. Immunohistochemical analysis with antisera specific for deer tick virus identified numerous immunoreactive neurons, with prominent involvement of large neurons in the brain stem, cerebellum, basal ganglia, thalamus, and spinal cord. This case demonstrates that deer tick virus can be a cause of fatal encephalitis.

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DEER TICK VIRUS IS A MEMBER OF THE TICKBORNE ENCEPHALITIS GROUP of flaviviruses and is closely related to Powassan virus. Deer tick virus was first isolated from *Ixodes scapularis* ticks in 1997 in North America.[1] The complete sequence of the deer tick virus has been determined.[2] The viral genome is 10.8 kb in length and shares 84% nucleotide sequence identity and 94% amino acid sequence identity with the Powassan virus genome. The two viruses are antigenically related,[3] and it has been suggested that they share a common origin and represent two viral lineages related to Powassan virus in North America.[2] Ebel et al.[4] refer to deer tick virus as Powassan virus lineage II, and in this report we use the same terminology.

Several members of the tickborne encephalitis group of flaviviruses, including tickborne encephalitis virus and Powassan virus, cause encephalitis in humans and animals, with tickborne encephalitis virus causing the most serious outbreaks. These viruses are closely related antigenically and are found predominantly in the northern hemisphere. In Europe, tickborne encephalitis occurs mainly in eastern and central regions and affects approximately 50 to 199 persons per 100,000 inhabitants annually.[5] The seroprevalence of antibodies to Powassan virus is estimated to be 0.5 to 4.0% in areas in which the disease is endemic.[6]

Infection with tickborne encephalitis virus can be mild or asymptomatic, or it can result in meningitis and encephalitis. Powassan virus can be pathogenic in human beings and can cause severe encephalitis with a fatality rate of up to 60% and long-term neurologic sequelae in survivors.[7] In contrast, Central European encephalitis that is caused by tick bites typically produces mild or silent infection. Other disease-causing flaviviruses include West Nile virus, St. Louis encephalitis virus, dengue virus, and yellow fever virus.[8] These viruses are transmitted by mosquitoes and cause a spectrum of diseases including meningitis, encephalitis, dengue fever, and yellow fever.

In certain locations of the northeastern and north central United States, the prevalence of deer tick virus in adult deer ticks is high,[9,10] but human infection has not been reported previously. This could indicate that the virus does not easily infect humans or that it is not particularly pathogenic. Diagnostic testing for Powassan virus is not routinely performed for patients with symptoms of encephalitis. Human incidence may thus be currently underestimated.

CASE REPORT

In late spring, a 62-year-old man was admitted to a local New York State hospital with a 4-day history of fatigue, fever, bilateral maculopapular palmar rash, and an onset of diplopia, dysarthria, and weakness in the right arm and leg. He was a native of New York State and had no history of recent travel. He owned horses and spent time outdoors in a wooded area. Reports of Lyme disease were common in his county of residence, indicating tick activity in the area. His medical history included chronic lymphocytic leukemia–small lymphocytic lymphoma (CLL–SLL), which had been diagnosed 4 years earlier and had initially been treated with fludarabine. He was not taking corticosteroids. On admission, he was given nonsteroidal antiinflammatory medication and an oral antibiotic (amoxicillin–clavulanate), which had been prescribed by his primary care physician for a recent exacerbation of chronic sinusitis that had been recurrent for more than a year. His baseline white-cell count was 15,000 cells per cubic millimeter and had increased to 70,000 cells per cubic millimeter during the past 6 to 8 months. He was started on broad-spectrum antibiotics and acyclovir (700 mg administered intravenously every 8 hours) for presumed infection of the central nervous system. The differential diagnosis included cerebral ischemia, possibly related to leukostasis, infection (viral, bacterial, or fungal), and lymphoma.

Initial laboratory results were notable for a markedly elevated peripheral-blood white-cell count (144,200 cells per cubic millimeter) and cerebrospinal fluid with normal glucose, minimally elevated protein, no white cells, and a negative Gram's stain (Table 1). The erythrocyte sedimentation rate was 4, blood cultures were sterile, and antibody titers were negative for *Borrelia burgdorferi* and *Anaplasma phagocytophilum*. The neurologic symptoms progressed, and after 2 days he was transferred to another hospital. At the time of transfer, the peripheral-blood white-cell count was 174,800 per cubic millimeter (with 4% neutrophils and 94% lymphocytes) (Table 1).

Findings on flow cytometry were characteristic of CLL–SLL. Bacterial and fungal blood cultures were sterile. Sputum cultures for tuberculosis and legionella species were negative. No serum antibodies to *Bartonella henselae* or leptospira or brucella species were detected. One day after admission, a repeat spinal tap showed an elevated protein level of 192 mg per deciliter, lymphocytic pleocytosis with 891 cells per cubic millimeter (with 1% neutrophils and 93% lymphocytes), and a normal glucose level (Table 1). Flow cytometry of the cerebrospinal fluid demonstrated a predominantly reactive T-cell population (98% of CD45+ cells were CD3+/CD5+ small T cells), with no evidence of CLL–SLL. Bacterial culture and Gram's staining of the cerebrospinal fluid were negative. India-ink staining, cryptococcus antigen test, and PCR analyses for herpes simplex virus types 1 and 2 and JC–BK virus were negative in cerebrospinal fluid.

Magnetic resonance imaging (MRI) performed after transfer (hospital day 1) revealed abnormal T_2-weighted and fluid-attenuated inversion recovery (FLAIR) images, with hyperintensities most prominent in the superior cerebellum, left pons, and bilateral basal ganglia (Fig. 1A, 1B, and 1C). An axial diffusion-weighted image and apparent-diffusion-coefficient sequences revealed restricted diffusion in the superior cerebellum, suggesting an ischemic process (Fig. 1D). The patient remained febrile (maximum temperature, 104.5°F [40.3°C]), and antimicrobial coverage was broadened to include an antifungal agent. His neurologic function deteriorated, which necessitated intubation, and his function did not improve despite maximal medical therapy.

On hospital day 4, his fever abated, and computed tomographic imaging revealed a mild obstructive hydrocephalus, leading to placement of an external ventricular drain. On hospital day 5, repeat MRI revealed worsening of signal abnormalities and markedly increased hydrocephalus. He was taken urgently to the operating room for decompression with a suboccipital craniotomy, at which time cerebellar biopsy was performed. Analysis of the biopsy specimen revealed severe meningoencephalitis with a dense meningeal lymphoid infiltrate containing mainly reactive CD4+ T cells, lymphocytic venous invasion and destruc-

tion, widespread loss of cerebellar Purkinje cells, occasional microglial nodules, and marked Bergmann gliosis (Fig. 1A to 1H in the Supplementary Appendix, available with the full text of this article at NEJM.org). The parenchyma was infiltrated by activated microglia–macrophages and predominantly CD8+ T cells (Fig. 1I and 1J in the Supplementary Appendix). All biopsy cultures were negative, and staining of biopsy tissue was negative for bacterial, fungal, and mycobacterial organisms and viral antigens (including herpes simplex virus 1 and 2, varicella–zoster virus, cytomegalovirus, influenza A, parainfluenza 3, adenovirus, and parvovirus).

MRI of the brain on hospital day 7 revealed progression of signal abnormalities, new lesions in the right thalamus and bilateral cerebral hemispheres, and persistent hydrocephalus (Fig. 2 in the Supplementary Appendix). By hospital day 11, there was no improvement in his status. Life support was withdrawn, and he died 17 days after the onset of symptoms. An autopsy was performed.

METHODS

CLINICAL SPECIMENS

A surgical biopsy of the cerebellum was fixed in formalin and embedded in paraffin. After autopsy, the brain was formalin-fixed for 2 weeks, and standard tissue blocks were paraffin-embedded. Unembedded, formalin-fixed brain tissue from the midbrain, cerebellum, pons, and spinal cord was submitted for PCR testing. (For details on viruses and control samples that were used, see the Methods section in the Supplementary Appendix.)

REVERSE-TRANSCRIPTASE–PCR AND SEQUENCE ANALYSIS

Nucleic acid was extracted from formalin-fixed tissue with the use of the WaxFree DNA extraction kit (TrimGen). This kit coextracts RNA. Ten microliters of extracted nucleic acid was reverse-transcribed to complementary DNA (cDNA) with the use of the iScript cDNA synthesis kit (Bio-Rad). Heminested reverse-transcriptase PCR (RT-PCR) for the detection of flavivirus with the use of universal primers was performed as described previously.[11,12] (In the Supplementary Appendix, additional information on the PCR primers is listed in Table A, and details regarding the PCR methods, sequence, and histologic and immunohistochemical analyses are listed in the Methods section.)

Table 1. Results of Analysis of Cerebrospinal Fluid and Blood of the Patient.*

Variable	First Hospitalization	Day 1 after Transfer to Second Hospital	Normal Range
Cerebrospinal fluid			
Glucose level (mg/dl)	59	47	40–70
Protein level (mg/dl)	64	192	15–45
White-cell count (cells/mm³)	0	891	0–5
Neutrophils (%)		1	0
Lymphocytes (%)		93	70
Complete blood count			
White-cell count (cells/mm³)	144,200	174,800	3500–9100
Neutrophils (%)	2	4	38–80
Lymphocytes (%)	98	94	15–40

* To convert the values for glucose to millimoles per liter, multiply by 0.05551.

RESULTS

The general autopsy revealed diffuse lymphadenopathy and splenomegaly and infiltration of liver and kidney by CLL–SLL. The brain weight was 1810 g (normal range in adults, 1300 to 1350), consistent with marked edema. On sectioning, there was marked softening and grayish discoloration throughout the brain stem and cerebellum.

Histologic examination of the brain revealed widespread meningopolioencephalitis and meningopoliomyelitis; there was no evidence of infiltration by CLL–SLL. A mild meningeal lymphocytic infiltrate persisted, and dense perivascular infiltrates were still identified in the parenchyma (Fig. 3C to 3K in the Supplementary Appendix). Throughout the brain, multinodular to patchy mononuclear infiltrates and confluent areas of necrosis were identified, along with microglial nodules and neuronophagia. This was most accentuated in large motor neurons of the brain stem (including cranial nerve nuclei), spinal anterior horns, cerebellum, basal ganglia, and thalamus (Fig. 2, and Fig. 3 in the Supplementary Appendix). Microglia–macrophage infiltration was greatest in gray-matter regions but also involved white-matter tracts to a lesser degree (Fig. 3A in the Supplementary Appendix).

As in the surgical biopsy, lymphocytic infiltrates in leptomeninges and perivascular spaces contained predominantly CD4+ helper T cells, whereas those in the parenchyma were predominantly CD8+ cytotoxic T cells (Fig. 4 in the Supplementary Appendix). CD8+ T cells were also

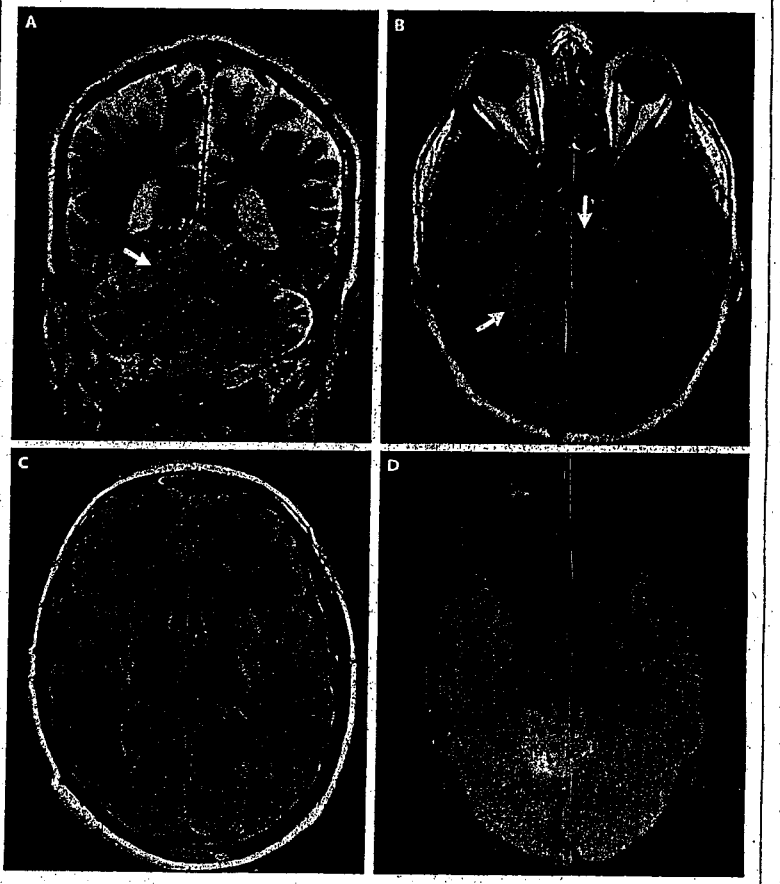

Figure 1. Magnetic Resonance Imaging (MRI) of the Brain on Hospital Admission.
MRI scanning that was performed on hospital day 1 revealed abnormal T_2-weighted signaling in the superior cerebellum (Panel A, arrow) and abnormal T_2-weighted fluid-attenuated inversion recovery images with hyperintensities in the cerebellum and left pons (Panel B, arrows) and in the bilateral basal ganglia (Panel C). The superior cerebellum was bright on diffusion-weighted imaging (Panel D) and dark on apparent-diffusion-coefficient sequences, which suggested an ischemic process.

more frequently identified in close apposition to surviving neurons (Fig. 2C, and Fig. 4A, 4B, and 4E in the Supplementary Appendix).

On the extracted nucleic acid from the formalin-fixed brain tissue, the following analyses were performed: a PCR panel including real-time PCR assays for the detection of herpes simplex viruses 1 and 2, Epstein–Barr virus, cytomegalovirus, human herpesvirus type 6, varicella–zoster virus, and adenovirus; real-time RT-PCR assays for the de-

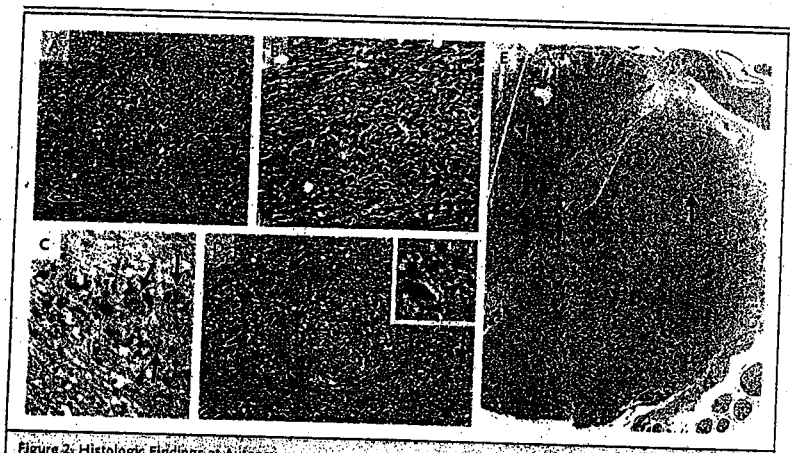

Figure 2. Histologic Findings at Autopsy.

In Panel A, microglial nodules and lymphocytic infiltrates in the pons are visible in basal pontine nuclei (arrowheads), with less prominent involvement of descending fiber tracts (arrow) and pontocerebellar fibers. In Panel B, confluent foci of parenchymal necrosis can be seen in pontine basal nuclei. In Panel C, CD8+ immunostaining of the basis pontis shows a cytotoxic T-cell infiltrate and a close association with surviving neurons (arrows). In Panel D, nearly complete neuronal loss is seen in the substantia nigra with rare surviving neurons (arrows); in the inset, an eosinophilic dying neuron and remaining neuromelanin pigment are engulfed in macrophages or free in the parenchyma (arrowheads). In Panel E, phosphoglucomutase 1 immunostaining of lumbar spinal cord shows marked infiltration by microglia–macrophages and in the anterior horn and focal microglial nodules in the lateral corticospinal tract (arrow) and posterior column (arrowhead). In Panels A, B, and D, paraffin sections were stained with hematoxylin and eosin.

tection of West Nile virus and eastern equine encephalitis virus; a real-time PCR assay using a cDNA template for the detection of enterovirus; a group-specific RT-PCR assay for the detection of alphaviruses[13]; and conventional PCR assays using a cDNA template for the detection of St. Louis encephalitis, California serogroup, and Cache Valley viruses. PCR assays for the detection of borrelia species, including *B. burgdorferi*, and of *A. phagocytophilum* were performed on DNA extracts from the cerebellum and spinal cord. All results were negative. A group-specific RT-PCR assay for the detection of flaviviruses gave PCR products of the expected size for both the first-round PCR and the nested PCR.[11] The PCR products of approximately 250 bp and 220 bp were purified from the gel and sequenced. A search with the use of the nucleotide Basic Local Alignment Search Tool (BLAST) algorithm posted on the Web site of the National Center for Biotechnology Information identified a 220-bp sequence sharing 97% of the sequence of deer tick virus strains CTB30 (accession number, AF311056.1), and IPS001 (accession number, AF310947.1) and Powassan virus strain R59266 (accession number, AF310948.1). To confirm the lineage of the virus, sequencing was performed with the use of previously published and newly designed primer sets from the envelope coding region, NS5, and sequences in the 3′ untranslated region[1,4] (Table A in the Supplementary Appendix).

With a total of 23 primer sets used, two regions of the virus were sequenced: 2748 bp, spanning part of the RNA polymerase coding sequence and the 3′ untranslated region of the virus, and 1180 bp of the envelope coding sequence. Phylogenetic analyses of these fragments indicated that the virus, named DT-NY-07, was most closely related to the deer tick virus (Fig. 3).[14-16]

To confirm that deer tick virus antigens were detectable in brain tissue from the patient, two polyclonal mouse antibody reagents were generated against whole deer tick virus and recombinant deer tick virus E protein (rEDTV). Both antiserum samples showed similar immunohistochemical specificity in both the cerebellar biopsy and autopsy specimens, although generally a larger number of neurons and viral antigens in macrophages were labeled with the whole-virus serum (Fig. 4, and Fig. 5 in the Supplementary Appendix). The whole-virus antiserum labeled neuronal-cell bodies, dendrites, and axons. The rEDTV serum and rarely the whole-virus serum also labeled rounded, granular-to-tubular profiles within the neuronal cytoplasm of large motor neurons, with a cellular distribution highly reminiscent of the Golgi apparatus in some neurons (Fig. 4A, and Fig. 6 in the Supplementary Appendix). Alternatively, the structures may represent viral particles within the lysosomal–endosomal system. A segmental distribution of labeled neurons was prominent in the hippocampus (Fig. 4B). In isocortical regions, occasional labeled neurons and a focus of infected cells consistent with oligodendrocytes were also identified (Fig. 4D).

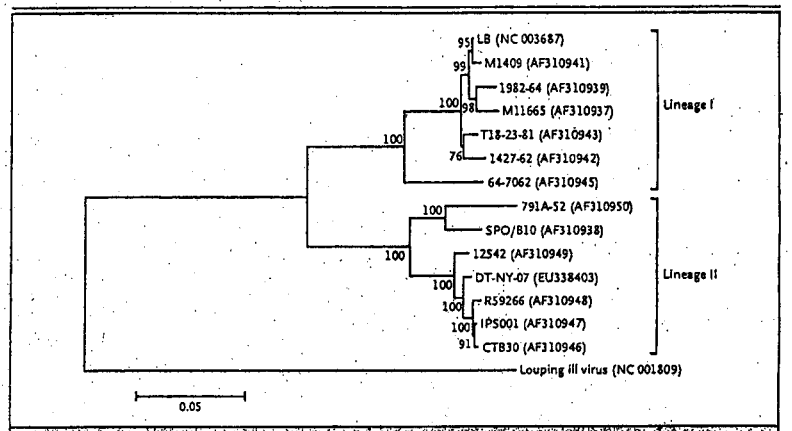

Figure 3. Phylogenetic Tree Showing the Relationship between the Virus (DT-NY-07) Detected in Tissue Sections from the Brain of the Patient and Other Powassan Viruses.

This phylogenetic tree was constructed from 2304 nucleotide sequences of the NS5 region; GenBank accession numbers are in parentheses. The evolutionary history was inferred with the use of the neighbor-joining method.[14] The optimal tree with the sum of branch length equaling 0.60849744 is shown. The percentage of replicate trees in which the associated taxa are clustered together in the bootstrap test (1000 replicates) is shown next to each branch. The tree is drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to construct the phylogenetic tree. To root the dendrogram, louping ill virus was used as the outgroup. The evolutionary distances were computed with the use of the maximum composite likelihood method[15] and are expressed in the units of the number of base substitutions per site. All positions containing gaps and missing data were eliminated from the data set. Phylogenetic analyses were conducted with the use of Molecular Evolutionary Genetics Analysis (MEGA) software, version 4.0.[16]

DISCUSSION

Strains of Powassan virus lineages I and II are distinct and are maintained in separate enzootic cycles because of differences in transmission vectors and geographic distribution. Lineage I strains are transmitted by ticks and have been reported in North America (mainly in New York State and Canada) and in eastern Russia, whereas lineage II strains have been isolated in the Atlantic Coast of the United States and in Wisconsin.[4] Lineage I strains appear to be associated with *I. cookei* and groundhogs (*Marmota monax*), whereas lineage II strains are associated with deer ticks and white-footed mice (*Peromyscus leucopus*).[7] In addition, lineage II strains have not previously been associated with human disease, whereas a number of infections in humans associated with lineage I strains have been documented.[17-21] From these re-

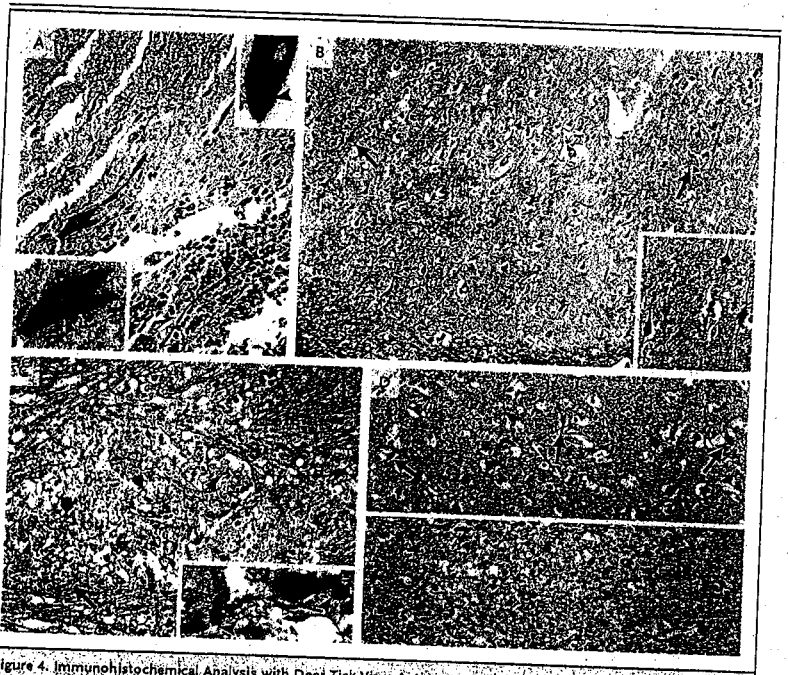

Figure 4. Immunohistochemical Analysis with Deer Tick Virus Antiserum Samples.

Paraffin sections of cerebellar samples obtained from the patient on biopsy (Panel A) and samples from the hippocampus (Panel B), pons (Panel C), and temporal cortex (Panel D), obtained at autopsy were stained either with antibody against whole deer tick virus (Panel A, upper inset, and Panels B and C) or with antibody against recombinant deer tick virus E protein (rEDTV) (Panel A, Panel A, lower inset, and Panel D). In Panel A, in the cerebellar biopsy sample, both types of antiserum recognized surviving Purkinje cells, with prominent filling of their dendrites in the molecular layer and occasional identification of axons in the granule-cell layer (arrow); in the insets, several Purkinje cells were identified with immunoreactive granular-to-tubular profiles (arrowheads). In Panel B, many hippocampal pyramidal neurons were immunolabeled in a segmental distribution (in area surrounding arrows), with prominent decoration of apical and basal processes (inset). In Panel C, many surviving immunolabeled neurons in the basis pontis are visible. The whole deer tick virus antibody also recognized viral antigens engulfed in macrophages (arrow; inset, arrowheads), whereas the rEDTV antibody did not have such recognition. In Panel D, in temporal cortex, immunoreactive neurons that were not associated with inflammatory reaction were occasionally identified (upper panel, arrows). In the temporal white matter, a focus of labeled cells consistent with oligodendrocytes was seen (lower panel). (For more details, see Fig. 5 and 6 in the Supplementary Appendix.)

ports, it appears that lineage I Powassan encephalitis is characterized by respiratory distress, fever, vomiting, convulsions, and occasionally paralysis.[17,19] Studies in the northern Ontario region of Canada show an antibody prevalence rate of as much as 3.2%, indicating that infection does not always cause severe disease.[22] In a phylogenetic study of Powassan-related viruses of North America, a lineage II strain (ON97) was reportedly isolated from human brain tissue.[2] However, no other information regarding the case was provided.

Confirmation of infection with a lineage I strain of Powassan virus has been made principally by serologic methods. Because of serologic cross-reactivity, these methods do not necessarily distinguish lineage I from lineage II strains. Neutralization assays are required for confirmation; molecular detection and sequence determination, as performed in our investigation, allowed for definitive classification of the virus.

In this study, we detected deer tick virus by both molecular and immunohistochemical methods in the central nervous system of a patient with encephalitis. The neurotropism seen in this case, with involvement of both gray and white matter, matches the pattern of central nervous system infection for arboviruses, which may be highly neuroinvasive.[23]

The patient was known to have frequented wooded areas, although no specific contact with ticks had been reported. He presented in late spring, which suggested that transmission was probably from nymphal deer ticks, which are most active during spring and summer months. In addition, since nymphal deer ticks are small in size (1.5 mm in diameter), it is not uncommon for their bites to remain undetected. It is possible that the patient's underlying condition (CLL–SLL) predisposed him to particularly serious disease. Reports of elderly and immunocompromised patients being at a greater risk for severe encephalitis caused by West Nile virus are well documented.[24,25]

Our immunohistochemical studies with newly generated deer tick virus antibodies demonstrated prominent labeling of neuronal-cell bodies and their processes; a focus of apparent oligodendroglial infection was also identified (Fig. 4). In addition, some neurons contained rounded granular-to-tubular profiles. A segmental distribution of immunolabeling was evident in the hippocampus, as was seen in cerebellum infected by central European tickborne encephalitis virus, as described previously.[26] The parenchymal lymphocytic infiltrates in this case and in previous pathological studies of tickborne encephalitis virus[26,27] were predominantly CD8+ cytotoxic T cells, which were also seen in close apposition to surviving neurons, further indicating that immunologic mechanisms may have contributed to nerve-cell destruction in tickborne encephalitides.

Diagnostic testing for Powassan virus is not routinely performed in patients with encephalitis. More extensive testing for arboviruses, including Powassan virus, might reveal that arboviral infections are more widespread than previously reported. For Powassan virus, testing is especially important during the summer months and in regions where infected ticks are prevalent. Deer ticks transmit several tickborne diseases, including Lyme disease, human babesiosis, and human granulocytic anaplasmosis.[28] This report of deer tick virus resulting in a fatal case of encephalitis emphasizes the significance of deer ticks in transmitting a variety of infections. There are limited data on the prevalence of infection with deer tick virus among adult deer ticks, although a rate of 0.6 to 1.3% in limited geographic areas in the United States has been reported.[9] Because no specific antiviral therapy is available for Powassan infection, the best strategy remains prevention (i.e., avoidance of contact with the arthropod vector). Studies to elucidate the prevalence and relative pathogenic features of Powassan lineages I and II are warranted.

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