



Published in final edited form as:

Clin Infect Dis. 2019 July 18; 69(3): 450–458. doi:10.1093/cid/ciy923.

Transmission of Eastern Equine Encephalitis Virus from an Organ Donor to Three Transplant Recipients

Stephanie M. Pouch, M.D., M.S.¹, Shalika B. Katugaha, M.D.², Wun-Ju Shieh, M.D., M.P.H., Ph.D.³, Pallavi Annambhotla, Dr.PH.⁴, William L. Walker, D.V.M., Ph.D.^{5,6}, Sridhar V. Basavaraju, M.D.⁴, Jefferson Jones, M.D., M.P.H.⁴, Thanhthao Huynh, D.V.M.³, Sarah Reagan-Steiner, M.D., M.P.H.³, Julu Bhatnagar, Ph.D.³, Kacie Grimm⁷, Susan L. Stramer, Ph.D.⁷, Julie Gabel, D.V.M., M.P.H.⁸, G. Marshall Lyon, M.D., M.M.Sc.¹, Aneesh K. Mehta, M.D.¹, Prem Kandiah, M.D.⁹, David C. Neujahr, M.D.¹⁰, Jeffrey Javidfar, M.D.¹¹, Ram M. Subramanian, M.D., M.B.A.¹², Samir M. Parekh, M.D.¹², Palak Shah, M.D., M.S.¹³, Lauren Cooper, M.D., M.H.S.¹³, Mitchell A. Psocka, M.D., Ph.D.¹³, Rachel Radcliffe, D.V.M., M.P.H.¹⁴, Carl Williams, D.V.M.¹⁵, Sherif R. Zaki, M.D., Ph.D.³, J. Erin Staples, M.D., Ph.D.⁵, Marc Fischer, M.D., M.P.H.⁵, Amanda J. Panella, M.P.H.⁵, Robert S. Lanciotti, Ph.D.⁵, Janeen J. Laven⁵, Olga Kosoy, M.S.⁵, Ingrid B. Rabe, M.B.Ch.B., M.Med.⁵, Carolyn V. Gould, M.D., M.S.C.R.⁵ Eastern Equine Encephalitis Virus Transplant Transmission Investigation Team*

¹Division of Infectious Diseases, Emory University School of Medicine, Atlanta, GA

²Infectious Diseases Physicians, Inc., Inova Fairfax Hospital Heart and Vascular Institute, Falls Church, VA

³Division of High-Consequence Pathogens and Pathology, National Center for Emerging and Zoonotic Infectious Diseases, Centers for Disease Control and Prevention, Atlanta, GA

⁴Division of Healthcare Quality Promotion, National Center for Emerging and Zoonotic Infectious Diseases, Centers for Disease Control and Prevention, Atlanta, GA

⁵Division of Vector-Borne Diseases, National Center for Emerging and Zoonotic Infectious Diseases, Centers for Disease Control and Prevention, Fort Collins, CO

⁶Epidemic Intelligence Service, Center for Surveillance, Epidemiology and Laboratory Services, Centers for Disease Control and Prevention, Atlanta, GA

Corresponding Author: Stephanie M. Pouch, M.D., M.S., 101 Woodruff Circle, Woodruff Memorial Research Building, Suite 2101, Atlanta, GA 30322, 404-712-7988 (phone), 404-712-2278 (fax).

*The members of the Eastern Equine Encephalitis Virus Transplant Transmission Investigation Team are as follows: Andrew B. Adams, M.D., Ph.D., Linda J. Bogar, M.D., Adam Cochrane, PharmD., Shashank S. Desai, M.D., Ryan M. Ford, M.D., Christopher W. May, M.D., Hassan Nayer, M.D., Jennie P. Perryman, R.N., Ph.D., Preeti A. Reshamwala, M.D., Ramesh Singh, M.D., Daniel G. Tang, M.D., and Joel P. Wedd, M.D.

Disclaimer

The findings and conclusions in this report are those of the authors and do not necessarily represent the official position of the Centers for Disease Control and Prevention.

Potential Conflicts of Interest

Dr. Shah reports grants from Haemonetics, grants from Merck, grants from Medtronic, payments from NuPulse, and grants from AHA / Enduring Hearts Scientist Development Grant, all outside the submitted work. All remaining authors: No reported conflicts relevant to the content of this manuscript. All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest.

The findings of this investigation were presented in part at the 67th Annual Epidemic Intelligence Service Conference in Atlanta, GA on April 17, 2018.

⁷American Red Cross, Gaithersburg, MD

⁸Georgia Department of Public Health, Atlanta, GA

⁹Department of Neurology and Neurosurgery, Emory University School of Medicine, Atlanta, GA

¹⁰Division of Pulmonary Allergy and Critical Care Medicine, Emory University School of Medicine, Atlanta, GA

¹¹Department of Surgery, Emory University School of Medicine, Atlanta, GA

¹²Division of Digestive Diseases, Emory University School of Medicine, Atlanta, GA

¹³Department of Heart Failure and Transplantation, Inova Fairfax Heart and Vascular Institute, Falls Church, VA

¹⁴Division of Acute Disease Epidemiology, South Carolina Department of Health and Environmental Control, Columbia, SC

¹⁵North Carolina Division of Public Health, Raleigh, NC

Abstract

Background—In fall 2017, three solid organ transplant recipients from a common donor developed encephalitis within one week of transplantation, prompting suspicion of transplant-transmitted infection. Eastern equine encephalitis virus (EEEV) infection was identified during testing of endomyocardial tissue from the heart recipient.

Methods—We reviewed medical records of the organ donor and transplant recipients and tested serum, whole blood, cerebrospinal fluid, and tissue from the donor and recipients for evidence of EEEV infection by multiple assays. We investigated blood transfusion as a possible source of organ donor infection by testing remaining components and serum specimens from blood donors. We reviewed data from the pre-transplant organ donor evaluation and local EEEV surveillance.

Results—We found laboratory evidence of recent EEEV infection in all organ recipients and the common donor. Serum collected from the organ donor upon hospital admission tested negative, but subsequent samples obtained prior to organ recovery were positive for EEEV RNA. There was no evidence of EEEV infection among donors of the eight blood products transfused into the organ donor or in products derived from these donations. Veterinary and mosquito surveillance showed recent EEEV activity in counties nearby the organ donor's county of residence. Neuroinvasive EEEV infection directly contributed to the death of one organ recipient and likely contributed to death in another.

Conclusions—Our investigation demonstrated EEEV transmission through solid organ transplantation. Mosquito-borne transmission of EEEV to the organ donor was the likely source of infection. Clinicians should be aware of EEEV as a cause of transplant-associated encephalitis.

Summary:

We describe the first reported transmission of eastern equine encephalitis virus (EEEV) infection through solid organ transplantation. The organ donor was likely infected with EEEV via mosquito-borne transmission. Clinicians should be aware of EEEV as a cause of transplant-associated encephalitis.

Keywords

eastern equine encephalitis virus; encephalitis; solid organ transplantation; donor-derived infection; transplant transmission

Eastern equine encephalitis virus (EEEV) is an arbovirus of the genus *Alphavirus*, family *Togaviridae*. The virus cycles between *Culiseta melanura* mosquitoes and birds residing in freshwater hardwood swamps; incidental transmission to other vertebrate species occurs through bridging vectors such as *Coquillettidia* and *Aedes* species mosquitoes [1-3]. Human cases occur sporadically or in small outbreaks, usually within close proximity to swamps or marshlands in the eastern United States [1, 2, 4]. While rare, EEEV causes one of the most severe arboviral diseases in North America, with approximately 30% fatality among reported cases [4-9].

EEEV disease in humans is a nationally-notifiable condition in the United States. State health departments report cases to ArboNET, the national arboviral disease surveillance system, using standardized case definitions [10]. From 2003–2016, a median of eight EEEV human neuroinvasive disease cases were reported annually [9]. Human disease cases were reported from 20 states, predominantly along the Atlantic and Gulf coasts. Non-human arboviral surveillance varies by jurisdiction, and reporting is voluntary.

While transplant and transfusion transmission of other arboviruses has been documented [11-15], to our knowledge, these modes of transmission have not been reported for EEEV. In fall 2017, three organ transplant recipients from a single donor developed fever and encephalitis approximately one week following transplantation. After initial testing for other infectious causes of encephalitis, EEEV transmission through organ transplantation was identified. We describe the investigation of these cases.

Methods

Investigation of the Organ Donor and Organ Transplant Recipients

Recognition of encephalitis in liver and lung recipients transplanted at the same center and from a common donor raised immediate concern for donor-derived infection. Medical personnel from this center contacted the organ procurement organization (OPO) to identify all organs and tissues recovered from the donor. The heart transplant recipient, who also had symptoms of encephalitis, was identified at another institution. Medical records and results of a pre-transplant interview with the organ donor's family were reviewed to identify any preceding febrile or neurologic illness and potential sources of pathogen exposure. Specific dates and locations are not provided in patient descriptions in order to maintain privacy.

As part of the investigation for causes of transplant-transmitted encephalitis, formalin-fixed paraffin-embedded (FFPE) tissue was obtained from a routine post-transplant endomyocardial biopsy from the heart recipient and tested at the Centers for Disease Control and Prevention (CDC) by immunohistochemical (IHC) staining for *Toxoplasma gondii*, microsporidia, flaviviruses, herpes viruses, and rabies virus. Histopathologic findings in the tissue prompted further testing for EEEV by IHC and EEEV-specific reverse-transcriptase

polymerase chain reaction (RT-PCR) assay to detect ribonucleic acid (RNA), followed by sequencing of PCR amplicons to confirm infection. Serum, whole blood, and cerebrospinal fluid (CSF) from all three organ transplant recipients were tested for evidence of EEEV infection using enzyme immunoassay (EIA) to detect immunoglobulin M (IgM) antibodies, plaque reduction neutralization test (PRNT) to confirm EEEV-specific neutralizing antibodies, and RT-PCR for RNA. Stored donor serum was tested for EEEV using both IgM EIA and RT-PCR, and a lymph node aspirate obtained for human leukocyte antigen typing was tested by IgM EIA and RT-PCR.

Investigation of the Source of Organ Donor EEEV Infection

We reviewed records of blood products received by the organ donor and identified the blood donors, other products derived from these donations (“co-components”), and recipients of transfused co-components. All remaining blood products available for retrieval, including samples from tubing attached to the original collection bag of transfused units (“retention segments”), and co-components were tested for EEEV IgM antibodies by EIA and RNA by RT-PCR. The blood supplier and local health departments contacted all respective blood donors; donors were asked about the presence of signs and symptoms consistent with EEEV infection and were offered follow up EEEV testing. We also reviewed human and non-human EEEV surveillance data reported by state health departments to ArboNET from within and around the organ donor’s county of residence in 2017.

Results

Organ Donor

A woman in her 40s with a history of hypertension and stroke was emergently admitted to a hospital in fall 2017 after sustaining a gunshot wound to the head; she progressed to brain death by hospital day one. Three organs (heart, liver, lungs) were recovered on day three of hospitalization after she received eight units of blood components. The donor’s family reported no recent febrile illness or neurologic symptoms. Standard laboratory screening for infectious pathogens conducted by the OPO prior to organ procurement revealed no evidence of active infection. Stored serum obtained on hospital day one, after transfusion of six units of blood components, showed no detectable EEEV RNA or IgM antibodies (Table 1). However, serum samples obtained on days two and three had detectable EEEV RNA but no EEEV IgM antibodies. A lymph node aspirate obtained prior to the transplant showed no evidence of EEEV infection.

Heart Recipient

A woman in her 40s with a history of breast cancer complicated by non-ischemic cardiomyopathy related to doxorubicin toxicity underwent orthotopic heart transplantation in fall 2017 from the common donor. Seven days after transplantation, she developed headache, hypotension, persistent fever, and progressive altered mental status (Figure 1). On the same day, prior to the onset of symptoms, an endomyocardial biopsy was performed according to routine protocol; the tissue revealed multiple foci of myocyte necrosis (Figure 2). EEEV antigens were detected by IHC in the tissue, and RT-PCR and sequencing of positive amplicons confirmed the presence of EEEV RNA (Table 1). Testing of the tissue for other

encephalitic pathogens was negative. CSF obtained nine days after transplantation revealed a neutrophilic pleocytosis (Table 2) and was positive for EEEV IgM and neutralizing antibodies but negative for EEEV RNA. Serum was negative for EEEV RNA and IgM antibodies but had detectable EEEV neutralizing antibodies. Magnetic resonance imaging (MRI) of the brain 11 days after transplantation demonstrated patchy T2-weighted and fluid-attenuated inversion recovery (T2/FLAIR) signal abnormality and edema involving the bilateral basal ganglia, thalami, and brainstem. She received multiple courses of intravenous immunoglobulin (IVIG) dosed at 0.4 g/kg/day and tapering doses of methylprednisolone for fevers and altered mental status. Serum collected 24 days after transplantation was positive for EEEV IgM and neutralizing antibodies. She had slow neurologic recovery but was eventually able to speak and participate in physical therapy; however, a subsequent endomyocardial biopsy demonstrated myocardial inflammation and necrosis consistent with either acute cellular rejection of the cardiac allograft or myocarditis. Despite treatment with antithymocyte globulin and methylprednisolone, she continued to have progressive heart failure and developed tachyarrhythmias related to ongoing myocardial injury. Multiple endomyocardial biopsies obtained over the course of six weeks showed persistent foci of myocyte necrosis with minimal neutrophilic and mononuclear infiltration. EEEV antigen and RNA remained detectable in the biopsies; however, no virions were observed on electron microscopy. The patient received three additional five-day courses of IVIG and methylprednisolone, but her graft failure progressed. She was transferred to another hospital three months after transplantation for evaluation of total artificial heart placement as a bridge to retransplantation. She subsequently developed sepsis due to possible intestinal perforation and was placed on extracorporeal membrane oxygenation but died approximately four months after transplantation.

Liver Recipient

A woman in her 40s with a history of autoimmune hepatitis and chronic pancytopenia underwent initial orthotopic liver transplantation (OLT) in 2015. Her course was complicated by acute cellular rejection and recurrence of autoimmune hepatitis, and she underwent second OLT from the common donor in fall 2017. One day after transplantation, she developed fevers to 40°C despite broad-spectrum antimicrobials, and five days later, she experienced confusion, which progressed to obtundation requiring intubation (Figure 1). MRI of the brain seven days after transplantation demonstrated non-enhancing lesions with restricted diffusion in the anterior bilateral medial temporal lobes, thalami, and basal ganglia; by ten days after transplantation, these lesions had progressed to involve the left caudate nucleus, hippocampi, midbrain, and cerebellum (Figure 3). CSF obtained eight days after transplantation showed a monocytic pleocytosis (Table 2) with detectable EEEV IgM antibodies but not EEEV RNA (Table 1). Serum was positive for EEEV IgM and neutralizing antibodies, and EEEV RNA was detected in whole blood. The patient received five days of IVIG (0.4 g/kg/day) and defervesced. She displayed minimal neurologic recovery, and her course was further complicated by chronic respiratory failure and suspected post-viral central nervous system or cerebral vasculitis (Figure 4), for which she underwent five rounds of plasmapheresis. Given her poor neurologic prognosis, care was withdrawn, and the patient died three months after transplantation.

Lung Recipient

A woman in her 50s with end-stage chronic obstructive pulmonary disease underwent bilateral lung transplantation from the common donor. Her post-operative course was notable for persistent neutrophilic leukocytosis. Seven days after transplantation, she developed fever (38.1°C) accompanied by altered mental status with an inability to follow commands (Figure 1). The following day, she demonstrated progressive altered mental status with a left-sided gaze preference and myoclonus, requiring endotracheal intubation for airway protection. Brain MRI performed 12 days after transplantation showed multifocal signals involving the brainstem, bilateral deep gray matter, bilateral hippocampi and medial temporal lobes, and left periaxial white matter concerning for meningoencephalitis on T2/FLAIR images. CSF obtained nine days after transplantation revealed a monocytic pleocytosis (Table 2), but limited sample volume precluded EEEV testing. A serum sample collected 11 days after transplantation was positive for EEEV IgM and neutralizing antibodies (Table 1). EEEV RNA was detected in whole blood. The patient received five days of IVIG (0.4 g/kg/day) and defervesced. Her course was further complicated by chronic respiratory failure requiring tracheostomy placement. Her neurological condition improved, and while left with a residual tremor, she was cognitively intact and began ambulating with physical therapy three months following transplantation.

Evaluation of the Source of Organ Donor EEEV Infection

The organ donor received three units of packed red blood cells, four units of fresh frozen plasma, and one unit of platelets from eight donors prior to organ recovery. Eighteen components were derived from the eight donors; eight components were transfused into the organ donor, three were transfused into other individuals, three were retrieved for EEEV testing, and four were discarded or unavailable. Retention segments of four transfused red blood cell components, three to the organ donor and one to another patient, were also retrieved for testing. Of the three recipients of co-components, two were discharged without symptoms and one died of underlying malignancy with no reported symptoms of encephalitis. Five of the blood donors responded to interview by the blood supplier and reported no febrile illness during the two weeks following donation. Four of the blood donors provided follow-up serum specimens. There was no evidence of EEEV infection in the eight donors by molecular and serologic testing of at least one donated component (n=4), serologic testing of a convalescent specimen (n=3), or both (n=1).

No recent human cases of EEEV disease in or around the donor's county of residence were reported to ArboNET. However, EEEV disease cases in horses and one positive mosquito pool were identified in nearby counties within an approximate 75 mile radius in the three months prior to her death [16].

Discussion

This investigation demonstrates the first known transmission of EEEV through solid organ transplantation; all three patients who received organs from a common donor developed EEEV neuroinvasive disease within one week of transplantation. Ultimately, two of the

patients died of complications likely related to EEEV, and one patient survived with residual neurologic deficits.

Most persons infected with EEEV are asymptomatic or have a non-specific mild illness [1]. Symptomatic persons typically develop a systemic febrile illness which can progress in <5% of individuals to neurologic disease, including headache, confusion, focal neurologic deficits, meningismus, seizures, or coma [6, 7, 17-19]. Survivors of EEEV encephalitis often have neurologic sequelae, such as seizure disorders, hemiplegia, and cognitive dysfunction [8, 17]. The fact that all three solid organ transplant recipients in this cohort developed encephalitis suggests that the risk of neuroinvasive disease may be increased with this route of transmission and/or in immunosuppressed patients. Little is known about risk of EEEV disease in immunocompromised patients; however, one case report of fatal EEEV encephalitis in a patient receiving rituximab was recently published [20].

All three organ recipients became symptomatic between six and seven days after transplantation, consistent with a reported EEEV incubation period of four to ten days [21, 22]. Similar to previous case reports of EEEV neuroinvasive disease in non-immunocompromised hosts, MRIs from all patients in this series demonstrated T2/FLAIR enhancement of the basal ganglia, thalami, and brain stem, findings which may assist in distinguishing EEEV from herpes simplex encephalitis [6, 23]. However, other common findings of EEEV, including peripheral leukocytosis, CSF neutrophilic pleocytosis, and absent hypoglycorrhachia, were variable among the three organ transplant recipients [6, 7, 24].

There is no human vaccine or specific antiviral therapy available for the treatment of EEEV, and management is supportive. All three organ recipients received at least five days of IVIG (0.4 g/kg/day) based on case reports describing its successful use in patients with EEEV encephalitis [25-27]. However, there are no controlled studies showing a proven benefit of IVIG in the management of EEEV infection.

Serum EEEV IgM antibodies typically appear within the first week of clinical illness. Since serologic testing during the acute phase of illness may be negative, convalescent serum should be tested for EEEV IgM if the diagnosis remains uncertain [28-30]. Despite being immunocompromised, all organ recipients demonstrated serologic evidence of recent EEEV infection in CSF and/or serum when sampled at least eight days after transplantation. Viremia occurs early in the course of EEEV-related illness and typically resolves by the onset of neurologic symptoms, limiting the utility of molecular testing of serum [31]. EEEV RT-PCR was negative in serum from all three transplant recipients and in CSF from two of the patients tested in this cohort, but the lung and liver recipients had detectable EEEV RNA in whole blood samples collected on the same day or two days after serum collection, respectively (Table 1). This suggests that, while RT-PCR testing of whole blood has not been evaluated for EEEV, it may have a higher probability of being positive than serum or CSF in patients with EEEV infection.

Detection of EEEV viral antigens and RNA by IHC and RT-PCR, respectively, in the heart transplant recipient's endomyocardial tissue suggests extraneural tissue involvement of

EEEV. Murine models have described the mesenchymal cell tropism of EEEV, characterized by early replication in peripheral tissues, including bones, skeletal muscle, tendons, and ventricular myocardium [32, 33]. Infection of the lungs, kidneys, stomach, and spleen have also been described in equine EEEV infection [34]. While cardiac sequelae of EEEV have infrequently been described in humans [8], EEEV-associated cytopathic effects in necrotic cardiac myocytes have been reported at equine and swine necropsy [34, 35] and from previous human autopsy samples (manuscript in preparation, CDC). The presence of myocyte necrosis with persistent EEEV antigen and RNA detection in endomyocardial tissue samples extending weeks after transplantation suggests that ongoing viral infection might have contributed to the patient's graft failure and ultimate death, although ongoing rejection was also suspected.

Transmission of EEEV likely occurred following infection in the organ donor via mosquito bites. The detection of EEEV RNA and lack of IgM antibodies in donor serum within 24 hours of organ procurement suggest that EEEV acquisition had occurred either shortly before or after hospitalization. However, there was no evidence of transfusion-transmission of EEEV infection from our investigation. Limited available surveillance data on equine disease cases and mosquito pools indicated EEEV activity in counties adjacent to the organ donor's residence. Nonetheless, we were unable to determine with certainty the source of EEEV infection in the donor because of limited details on epidemiologic risk factors prior to death, limited non-human surveillance data, and unknown sensitivity of testing blood samples from retention segments for EEEV infection.

In addition to EEEV described here, CDC has reported 15 other events of infectious encephalitis transmitted through solid organ transplantation since 2002, including West Nile virus, rabies virus, lymphocytic choriomeningitis virus, *Balamuthia mandrillaris*, and microsporidiosis [36]. Investigation of these transmission events has highlighted several important diagnostic and clinical challenges related to recognition and treatment of transplant-transmitted infections. Encephalitis resulting from transplant-transmitted pathogens, including EEEV, is rare and may not immediately be recognized by clinicians. In many instances, donors did not demonstrate clinical evidence of infection, and death was attributed to another cause, similar to the transmission event described here. Diagnosis is further complicated due to limitations with laboratory screening. No commercially available organ donor screening test is available for EEEV. However, if routine testing were implemented, there would be a risk of false positives because of low population prevalence, which could result in unnecessary organ discard. Finally, although there are no known effective prophylactic or treatment options for EEEV encephalitis, there is limited evidence that prophylaxis or treatment may be effective following exposure to some transplant-transmitted pathogens that cause encephalitis [36-39]. As a result, identification of possible infectious encephalitis among organ donors is crucial. To assist clinicians who may be evaluating donors with transmissible infectious encephalitis, efforts are underway to develop standardized risk assessment tools [40].

Clinicians should remain vigilant for clusters of encephalitis following solid organ transplantation. EEEV or other arboviral infections should be considered in recipients who develop encephalitis after transplantation, particularly if donors and recipients reside in

endemic areas of the United States. When donor-derived infection, including encephalitis, is suspected, providers must report the case to the United Network for Organ Sharing/Organ Procurement and Transplantation Network for investigation by the ad hoc Disease Transmission Advisory Committee and to public health authorities for expedited evaluation and identification of potentially infected organs and tissues.

Acknowledgments

We are indebted to Ibne Ali (CDC), Eileen Burd (Emory University), Jennifer Cope (CDC), Cynthia Goldsmith (CDC), Jeannette Guarner (Emory University), Amelia Keaton (Epidemic Intelligence Service), Colleen Kraft (Emory University), Jose Lima (American Red Cross), and Shawna Stuck (Georgia Department of Public Health) for their assistance in this investigation.

References

1. Davis LE, Beckham JD, Tyler KL. North American encephalitic arboviruses. *Neurol Clin* 2008; 26(3): 727–57. [PubMed: 18657724]
2. Goldfield M, Sussman O. The 1959 outbreak of Eastern encephalitis in New Jersey. I. Introduction and description of outbreak. *Am J Epidemiol* 1968; 87(1): 1–10. [PubMed: 5637871]
3. Armstrong PM, Andreadis TG. Eastern equine encephalitis virus--old enemy, new threat. *N Engl J Med* 2013; 368(18): 1670–3. [PubMed: 23635048]
4. Adams DA, Thomas KR, Jajosky RA, et al. Summary of Notifiable Infectious Diseases and Conditions - United States, 2015. *MMWR Morb Mortal Wkly Rep* 2017; 64(53): 1–143.
5. Reimann CA, Hayes EB, DiGuiseppi C, et al. Epidemiology of neuroinvasive arboviral disease in the United States, 1999–2007. *Am J Trop Med Hyg* 2008; 79(6): 974–9. [PubMed: 19052314]
6. Deresiewicz RL, Thaler SJ, Hsu L, Zamani AA. Clinical and neuroradiographic manifestations of eastern equine encephalitis. *N Engl J Med* 1997; 336(26): 1867–74. [PubMed: 9197215]
7. Przelomski MM, O'Rourke E, Grady GF, Berardi VP, Markley HG. Eastern equine encephalitis in Massachusetts: a report of 16 cases, 1970–1984. *Neurology* 1988; 38(5): 736–9. [PubMed: 3362371]
8. Ayres JC, Feemster RF. The sequelae of eastern equine encephalomyelitis. *N Engl J Med* 1949; 240(24): 960–2. [PubMed: 18144801]
9. Lindsey NP, Staples JE, Fischer MA. Eastern equine encephalitis in the United States, 2003–2016. *Am J Trop Med Hyg* 2018 3 19. doi: 10.4269/ajtmh.17-0927. [Epub ahead of print]
10. Centers for Disease Control and Prevention. Arboviral diseases, neuroinvasive and non-neuroinvasive: 2015 case definition. Available at <https://wwwn.cdc.gov/nndss/conditions/easternequine-encephalitis-virus-disease/case-definition/2015/>. Accessed October 18, 2017.
11. Iwamoto M, Jernigan DB, Guasch A, et al. Transmission of West Nile virus from an organ donor to four transplant recipients. *N Engl J Med* 2003; 348(22): 2196–203. [PubMed: 12773646]
12. Venkat H, Adams L, Sunenshine R, et al. St. Louis encephalitis virus possibly transmitted through blood transfusion-Arizona, 2015. *Transfusion* 2017; 57(12): 2987–94. [PubMed: 28905395]
13. Matos D, Tomashek KM, Perez-Padilla J, et al. Probable and possible transfusion-transmitted dengue associated with NS1 antigen-negative but RNA confirmed-positive red blood cells. *Transfusion* 2016; 56(1): 215–22. [PubMed: 26469514]
14. Motta IJ, Spencer BR, Cordeiro da Silva SG, et al. Evidence for transmission of Zika Virus by platelet transfusion. *N Engl J Med* 2016; 375(11): 1101–3. [PubMed: 27532622]
15. Pealer LN, Marfin AA, Petersen LR, et al. Transmission of West Nile virus through blood transfusion in the United States in 2002. *N Engl J Med* 2003; 349(13): 1236–45. [PubMed: 14500806]
16. Centers for Disease Control and Prevention. ArboNET disease maps. Available at https://wwwn.cdc.gov/arboNET/maps/ADB_Diseases_Map/index.html. Accessed March 28, 2018.

17. Letson GW, Bailey RE, Pearson J, Tsai TF. Eastern equine encephalitis (EEE): a description of the 1989 outbreak, recent epidemiologic trends, and the association of rainfall with EEE occurrence. *Am J Trop Med Hyg* 1993; 49(6): 677–85. [PubMed: 8279635]
18. Goldfield M, Welsh JN, Taylor BF. The 1959 outbreak of Eastern Encephalitis in New Jersey. 4. CF reactivity following overt and inapparent infection. *Am J Epidemiol* 1968; 87(1): 23–31. [PubMed: 5637874]
19. Clarke DH. Two nonfatal human infections with the virus of eastern encephalitis. *Am J Trop Med Hyg* 1961; 10: 67–70. [PubMed: 13693885]
20. Solomon IH, Ciarlino P, Santagata S, et al. Fatal eastern equine encephalitis in a patient on maintenance rituximab: a case report. *Open Forum Infect Dis* 2017; 4(1): ofx021. [PubMed: 28480291]
21. Sherwood JA, Oliver J. Eastern equine encephalitis incubation time periods of 5 and 8 days. *Pediatr Infect Dis J* 2015; 34(4): 459–60. [PubMed: 25760571]
22. Centers for Disease Control and Prevention. Technical fact sheet: eastern equine encephalitis. Available at <https://www.cdc.gov/easternequineencephalitis/tech/factsheet.html>. Accessed December 4, 2017.
23. Nickerson JP, Kannabiran S, Burbank HN. MRI findings in eastern equine encephalitis: the "parenthesis" sign. *Clin Imaging* 2016; 40(2): 222–3. [PubMed: 26995574]
24. Silverman MA, Misasi J, Smole S, et al. Eastern equine encephalitis in children, Massachusetts and New Hampshire, USA, 1970–2010. *Emerg Infect Dis* 2013; 19(2): 194–201. [PubMed: 23343480]
25. Wendell LC, Potter NS, Roth JL, Salloway SP, Thompson BB. Successful management of severe neuroinvasive eastern equine encephalitis. *Neurocrit Care* 2013; 19(1): 111–5. [PubMed: 23733173]
26. Golomb MR, Durand ML, Schaefer PW, McDonald CT, Maia M, Schwamm LH. A case of immunotherapy-responsive eastern equine encephalitis with diffusion-weighted imaging. *Neurology* 2001; 56(3): 420–1. [PubMed: 11171919]
27. Mukerji SS, Lam AD, Wilson MR. Eastern equine encephalitis treated with intravenous immunoglobulins. *Neurohospitalist* 2016; 6(1): 29–31. [PubMed: 26740855]
28. Sherwood JA, Brittain DC, Howard JJ, Oliver J. Antibody and viral nucleic acid testing of serum and cerebrospinal fluid for diagnosis of eastern equine encephalitis. *J Clin Microbiol* 2015; 53(8): 2768–72. [PubMed: 26063852]
29. Feemster RF. Outbreak of encephalitis in man due to the eastern virus of equine encephalomyelitis. *Am J Public Health Nations Health* 1938; 28(12): 1403–10. [PubMed: 18014957]
30. Gibney KB, Robinson S, Mutebi JP, et al. Eastern equine encephalitis: an emerging arboviral disease threat, Maine, 2009. *Vector Borne Zoonotic Dis* 2011; 11(6): 637–9. [PubMed: 21254938]
31. Carrera JP, Forrester N, Wang E, et al. Eastern equine encephalitis in Latin America. *N Engl J Med* 2013; 369(8): 732–44. [PubMed: 23964935]
32. Gardner CL, Burke CW, Tesfay MZ, Glass PJ, Klimstra WB, Ryman KD. Eastern and Venezuelan equine encephalitis viruses differ in their ability to infect dendritic cells and macrophages: impact of altered cell tropism on pathogenesis. *J Virol* 2008; 82(21): 10634–46. [PubMed: 18768986]
33. Vogel P, Kell WM, Fritz DL, Parker MD, Schoepp RJ. Early events in the pathogenesis of eastern equine encephalitis virus in mice. *Am J Pathol* 2005; 166(1): 159–71. [PubMed: 15632009]
34. Del Piero F, Wilkins PA, Dubovi EJ, Biolatti B, Cantile C. Clinical, pathologic, immunohistochemical, and virologic findings of eastern equine encephalomyelitis in two horses. *Vet Pathol* 2001; 38(4): 451–6. [PubMed: 11467481]
35. Elvinger F, Liggett AD, Tang KN, et al. Eastern equine encephalomyelitis virus infection in swine. *J Am Vet Med Assoc* 1994; 205(7): 1014–6. [PubMed: 7852154]
36. Basavaraju SV, Kuehnert MJ, Zaki SR, Sejvar JJ. Encephalitis caused by pathogens transmitted through organ transplants, United States, 2002–2013. *Emerg Infect Dis* 2014; 20(9): 1443–51. [PubMed: 25148201]
37. Smith RM, Muehlenbachs A, Schaenmann J, et al. Three cases of neurologic syndrome caused by donor-derived microsporidiosis. *Emerg Infect Dis* 2017; 23(3): 387–95. [PubMed: 28220747]
38. Vora NM, Basavaraju SV, Feldman KA, et al. Raccoon rabies virus variant transmission through solid organ transplantation. *JAMA* 2013; 310(4): 398–407. [PubMed: 23917290]

39. Mathur G, Yadav K, Ford B, et al. High clinical suspicion of donor-derived disease leads to timely recognition and early intervention to treat solid organ transplant-transmitted lymphocytic choriomeningitis virus. *Transpl Infect Dis* 2017; 19(4).
40. Smalley HK, Anand N, Buczek D, et al. Assessment of risk for transplant-transmissible infectious encephalitis among deceased organ donors. *Transpl Infect Dis* 2018: e12933. [PubMed: 29809311]

Author Manuscript

Author Manuscript

Author Manuscript

Author Manuscript

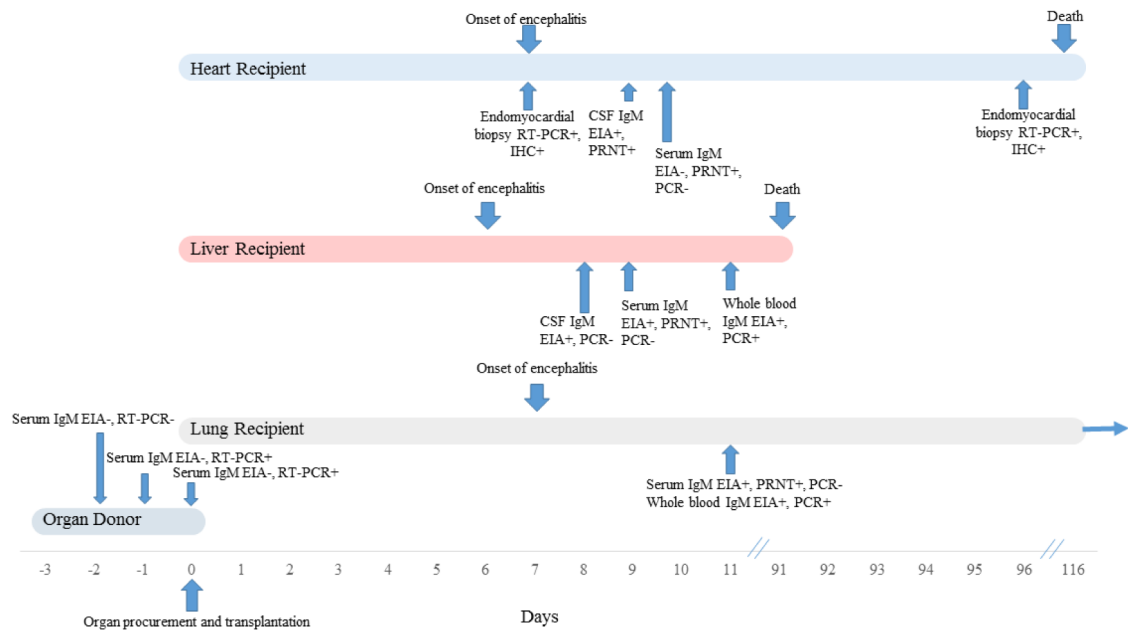


Figure 1. Clinical and Selected Laboratory Findings in the Organ Donor and Three Organ Transplant Recipients with Transplant-Transmitted Eastern Equine Encephalitis Virus Infection.

Tests for infection consisted of eastern equine encephalitis virus (EEEV)-specific serum, whole blood, and cerebrospinal fluid (CSF) immunoglobulin M enzyme immunoassay (IgM EIA), plaque reduction neutralization test (PRNT), and reverse-transcriptase polymerase chain reaction (RT-PCR). Formalin-fixed paraffin-embedded tissue was also evaluated for EEEV using immunohistochemistry (IHC) and RT-PCR. Plus signs denote positive results, and minus signs negative results.

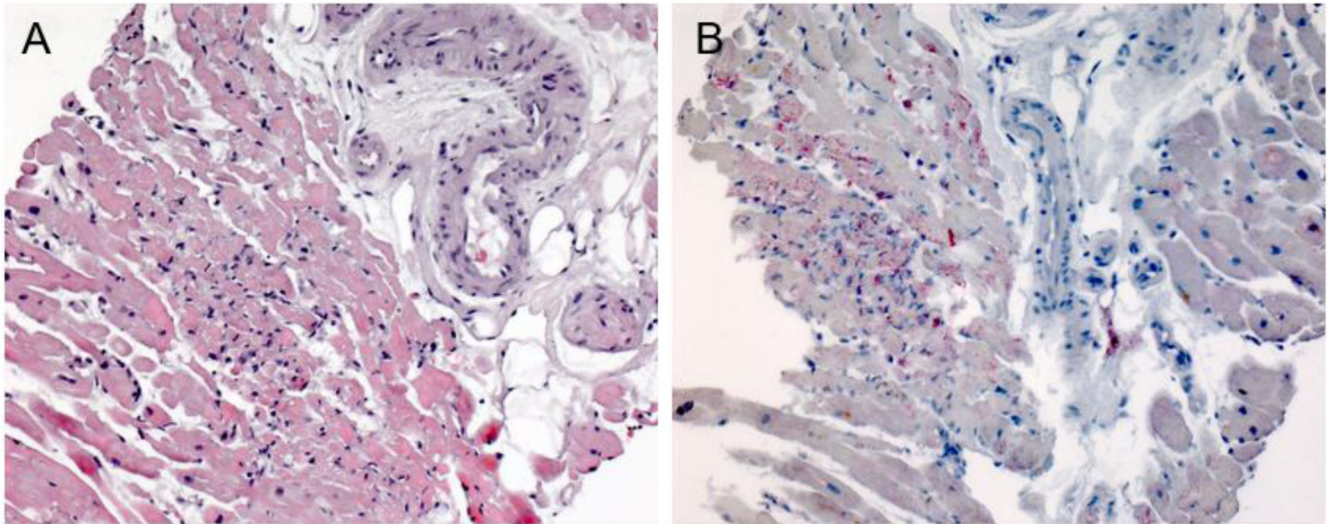


Figure 2. Histologic Findings and Immunohistochemical Evidence of Eastern Equine Encephalitis Virus in the First Endomyocardial Biopsy from the Heart Transplant Recipient (Obtained One Week after Transplantation).

Shown are foci of cardiomyocyte necrosis characterized by myofiber fragmentation, hypereosinophilia, and atrophy with karyorrhexis and minimal to absent inflammation (H&E, Panel A). Immunostaining of eastern equine encephalitis viral antigen is detected within areas of cardiomyocyte necrosis (EEEV IHC, Panel B).

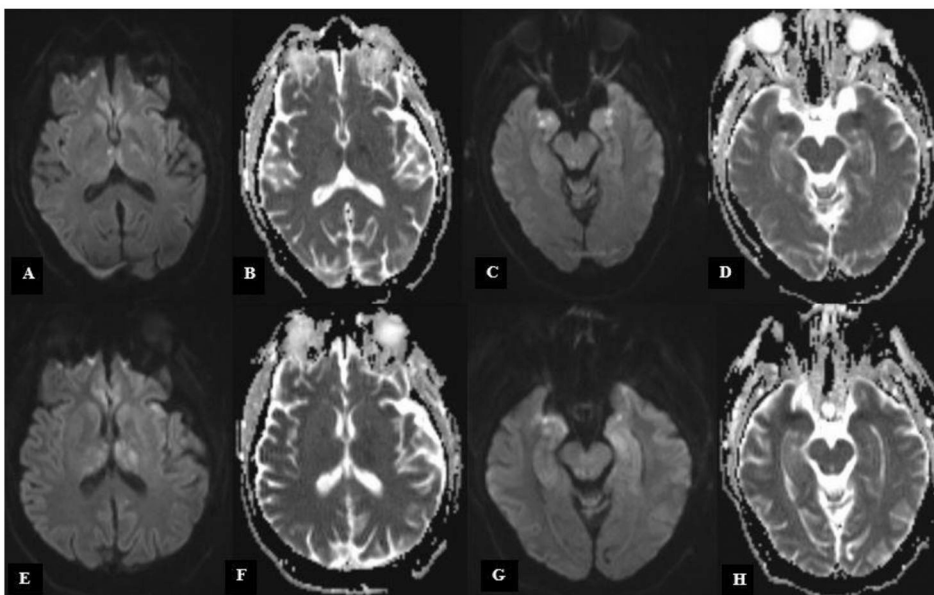


Figure 3. Diffusion-Weighted Imaging (DWI) and Apparent Diffusion Coefficient (ADC) Magnetic Resonance Imaging (MRI) Showing Progression of Intracranial Lesions in the Liver Recipient.

MRI on post-transplant day seven showed punctate areas of restricted diffusion in the bilateral thalami (DWI, Panel A and ADC, Panel B) and bilateral medial temporal lobes (DWI, Panel C and ADC, Panel D). MRI on post-transplant day ten showed an increase in the punctate areas of restricted diffusion in the bilateral thalami (DWI, Panel E and ADC, Panel F) and in the bilateral medial temporal lobes (DWI, Panel G and ADC, Panel H).

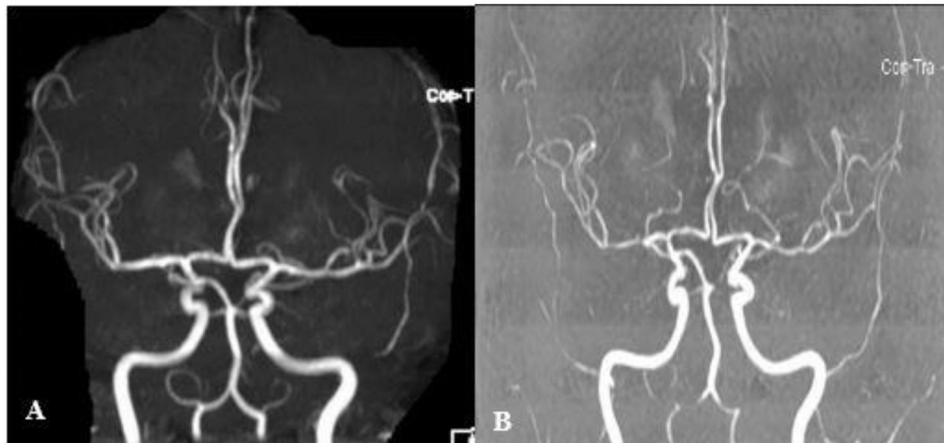


Figure 4. Magnetic Resonance Angiography Suggestive of Post-Viral Central Nervous System or Cerebral Vasculitis in the Liver Recipient.

Magnetic resonance angiography (MRA) was obtained on post-transplant day 36 (Panel A) and post-transplant day 46 (Panel B). Compared to Panel A, Panel B reveals interval development of significant multifocal narrowing of the bilateral anterior and posterior intracranial circulation concerning for vasculitis.

Table 1:

Clinical Characteristics and Eastern Equine Encephalitis Virus Testing Results in the Organ Donor and Three Transplant Recipients

Patient	Sex	Days from Transplantation to Symptom Onset	Days from Transplantation to Specimen Collection	Specimen Tested	EEEV Test Results	Disease Outcome
Organ Donor	F	N/A	-2	Stored serum	Negative IgM EIA, negative RT-PCR	Died of gunshot wound
			-1	Stored serum	Negative IgM EIA, positive RT-PCR	
			0	Stored serum	Negative IgM EIA, positive RT-PCR	
			0	Stored lymph node aspirate	Negative IgM EIA, negative RT-PCR	
Heart Recipient	F	7	-1	Stored pre-transplant serum	Negative IgM EIA	Developed heart failure related to EEEV myocarditis vs. acute rejection and died of sepsis during evaluation for re-transplantation
			7	Post-transplant endomyocardial biopsy	Positive RT-PCR, positive IHC	
			9	Post-transplant CSF	Positive IgM EIA, positive PRNT (1:4), negative RT-PCR	
			10	Post-transplant serum	Negative IgM EIA, positive PRNT (1:40), negative RT-PCR	
			12	Post-transplant serum	Negative IgM EIA, negative RT-PCR	
			24	Post-transplant serum	Positive IgM EIA, positive PRNT (1: 20,480), negative RT-PCR	
			40	Post-transplant endomyocardial biopsy	Positive RT-PCR, positive IHC	
			54	Post-transplant endomyocardial biopsy	Positive RT-PCR, positive IHC	
			67	Post-transplant endomyocardial biopsy	Positive RT-PCR, positive IHC; EM negative for virions	
			81	Post-transplant serum	Negative RT-PCR	
Liver Recipient	F	6	81	Post-transplant endomyocardial biopsy	Positive RT-PCR, positive IHC; EM negative for virions	Died of complications of EEEV encephalitis
			96	Post-transplant endomyocardial biopsy	Positive RT-PCR, positive IHC	
			101	Post-transplant CSF	Negative RT-PCR	
			8	Post-transplant CSF	Positive IgM EIA, negative RT-PCR	
			9	Post-transplant serum	Positive IgM EIA, positive PRNT (1:160), negative RT-PCR	

Patient	Sex	Days from Transplantation to Symptom Onset	Days from Transplantation to Specimen Collection	Specimen Tested	EEEV Test Results	Disease Outcome
Lung Recipient	F	7	11	Post-transplant whole blood	Positive IgM EIA, positive RT-PCR	Alive
			26	Post-transplant serum	Positive IgM EIA, negative RT-PCR	
			76	Post-transplant CSF	Negative RT-PCR	
			9	Post-transplant CSF	QNS	
			11	Post-transplant serum	Positive IgM EIA, positive PRNT (1:2560), negative RT-PCR	
			11	Post-transplant whole blood	Positive IgM EIA, positive RT-PCR	
			26	Post-transplant serum	Negative IgM EIA, negative RT-PCR	

F: female; CSF: cerebrospinal fluid; IgM EIA: Immunoglobulin M antibodies by enzyme immunoassay; RT-PCR: reverse transcriptase-polymerase chain reaction; EEEV: eastern equine encephalitis virus; PRNT: plaque reduction neutralization test; IHC: immunohistochemistry; QNS: quantity not sufficient for testing; EM: electron microscopy

Table 2:

Selected Laboratory Results for the Organ Transplant Recipients during Acute Neuroinvasive Eastern Equine Encephalitis Virus Disease

Variable	Heart Recipient	Liver Recipient	Lung Recipient
<i>Cerebrospinal Fluid</i>			
Appearance	Xanthochromic	Xanthochromic	Xanthochromic
White blood cell count (per μL)	217	88	10
Neutrophils (%)	66	32	5
Lymphocytes (%)	27	11	15
Monocytes (%)	7	57	80
Red blood cell count (per μL)	18000	2	9
Glucose (mg/dl)	38	120	83
Protein (mg/dl)	170	>200	78
Opening pressure (cm H_2O)	32	30	20
<i>Blood*</i>			
White blood cell count (per μL) ^{**}	12.7	3.5	30.5
Neutrophils (%)	ND	69	93
Lymphocytes (%)	ND	22	1
Monocytes (%)	ND	8	4
Hemoglobin (g/dl)	10.3	8.3	9.6
Platelet count (per μL)	221	27	103
Serum sodium (mmol/L)	127	127	149
Serum creatinine (mg/dl)	0.8	2.5	1.2
Aspartate aminotransferase (U/L)	42	44	24
Alanine aminotransferase (U/L)	40	47	30
Alkaline phosphatase (U/L)	67	211	62
Tacrolimus trough (ng/mL)	9.3	7.0	10.3

ND: not done

* Obtained on the day of onset of encephalitis

** Concomitant daily steroid doses for the organ recipients: Heart Recipient – 25mg prednisone; Liver Recipient – 20mg prednisone; Lung Recipient – 25mg methylprednisolone