

## Novel Clinical and Pathologic Findings in a Heartland Virus–Associated Death

Mary-Margaret A. Fill,<sup>1,2</sup> Margaret L. Compton,<sup>3</sup> Edward C. McDonald,<sup>3</sup> Abelardo C. Moncayo,<sup>2</sup> John R. Dunn,<sup>2</sup> William Schaffner,<sup>4</sup> Julu Bhatnagar,<sup>5</sup> Sherif R. Zaki,<sup>5</sup> Timothy F. Jones,<sup>2</sup> and Wun-Ju Shieh<sup>5</sup>

<sup>1</sup>Centers for Disease Control and Prevention, Epidemic Intelligence Service, Division of Scientific Education and Professional Development, Atlanta, Georgia; <sup>2</sup>Tennessee Department of Health, Division of Communicable and Environmental Diseases and Emergency Preparedness; <sup>3</sup>Vanderbilt University Medical Center, Department of Pathology, Microbiology, and Immunology, and <sup>4</sup>Vanderbilt University School of Medicine, Department of Health Policy, Nashville, Tennessee; and <sup>5</sup>Centers for Disease Control and Prevention, National Center for Emerging and Zoonotic Infectious Diseases, Infectious Diseases Pathology Branch, Atlanta, Georgia

We describe an investigation into a Heartland virus (HRTV)–associated death in Tennessee with novel clinical and pathologic findings. HRTV can cause rapidly fatal, widely disseminated infection with multisystem organ failure in patients without substantial comorbidities. We identified viral antigen in multiple organ tissues where it was not detected previously.

**Keywords.** emerging infections; tickborne disease; viral infections; heartland virus.

Heartland virus (HRTV) is an emerging tickborne phlebovirus [1]. Phylogenetic analysis has indicated that HRTV is closely related to severe fever with thrombocytopenia syndrome (SFTS) virus that has been isolated in Asia [1–3]. Clinical symptoms of HRTV infection include fever, fatigue, headache, nausea, diarrhea, and myalgia. Laboratory features include leukopenia, thrombocytopenia, and elevated liver enzyme studies [1, 4, 5]. Since 2009, 8 HRTV cases have been reported in the United States; all illnesses occurred among men aged  $\geq 50$  years, of whom 75% ( $n = 6/8$ ) required hospitalization and 88% ( $n = 7/8$ ) reported history of a tick bite [1, 4, 5]. One death occurred [5]. HRTV antigen has previously been detected in premortem bone marrow, whole blood and serum, and postmortem lymph node and splenic tissue [1, 5].

### CASE REPORT

In July 2015, a man aged 68 years with a past medical history of limited intracerebral hemorrhage, stage T2b melanoma, and

hypertension presented to a local emergency department with complaints of rash and pain in his left lower extremity. He reported a tick bite during the 2 weeks preceding illness onset. Laboratory evaluations were normal. He was prescribed doxycycline and discharged home. During subsequent days, he developed fever, progressive weakness, recurrent falls, nausea, vomiting, and confusion. He returned to the same emergency department 4 days later. Laboratory studies revealed thrombocytopenia to  $86 \times 10^3$  cells/ $\mu\text{L}$ , hyponatremia to 127 mEq/L, elevated aspartate aminotransferase (AST) to 99 U/L, and elevated alanine aminotransferase (ALT) to 73 U/L. The only acute process identified on chest radiography; computed tomography of the head, abdomen, and pelvis; and magnetic resonance imaging of the brain was mildly prominent left inguinal lymphadenopathy.

On hospital day 2, he was transferred to a regional medical center for further evaluation. Lumbar puncture showed cerebrospinal fluid (CSF) analysis to be within normal limits. He was treated with intravenous vancomycin and ceftriaxone. Because of worsening hyperbilirubinemia (total bilirubin, 7.8 mg/dL; direct bilirubin, 7.0 mg/dL) and liver function studies (AST, 996 U/L; ALT, 352 U/L), he underwent endoscopic retrograde cholangiopancreatography, which revealed no obstruction or infection source.

On hospital day 5, he was transferred to an academic medical center for septic shock, altered mental status, and acute renal failure. He remained intermittently febrile (maximum temperature 40.1°C), tachycardic, and hypotensive despite norepinephrine, phenylephrine, and vasopressin. Broad-spectrum antibiotic coverage was continued. Laboratory findings included leukocytosis ( $31.1 \times 10^3$  cells/ $\mu\text{L}$ ), thrombocytopenia ( $15 \times 10^3$  cells/ $\mu\text{L}$ ), coagulopathy (international normalized ratio, 1.8; fibrinogen, 89 mg/dL), increased creatine kinase (7361 IU/L), increased lactate dehydrogenase (5093 U/L), elevated creatinine (3.24 mg/dL), severe anion gap metabolic acidosis (bicarbonate, 11 mEq/L; lactate, 13.0 mg/dL), and markedly increased ferritin (46789 ng/mL). Multiple sets of blood, urine, and CSF cultures revealed no bacterial growth. Serology for spotted fever group *Rickettsia* species, *Ehrlichia* species, and *Borrelia burgdorferi* were negative. He tested negative for human immunodeficiency virus and hepatitis A, B, and C viruses.

His hospital course was complicated by severe septic shock requiring multiple vasopressors, acute respiratory distress syndrome requiring mechanical ventilation, disseminated intravascular coagulation, renal failure requiring continuous renal replacement therapy, atrial fibrillation with rapid ventricular response, and delirium. Despite maximal medical therapy, his clinical status continued to deteriorate and he died on hospital day 6.

Received 9 August 2016; editorial decision 28 October 2016; accepted 16 November 2016; published online January 4, 2017.

Correspondence: M.-M. A. Fill, 710 James Robertson Parkway, Andrew Johnson Tower, Fourth Floor, Nashville, TN 37243 (mary-margaret.fill@tn.gov).

Clinical Infectious Diseases® 2017;64(4):510–2

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DOI: 10.1093/cid/ciw766

## METHODS

A clinical records review was performed. A full autopsy was performed at the hospital. Formalin-fixed, paraffin-embedded (FFPE) tissues were obtained from all major organ systems. Serum was collected and fresh frozen tissue specimens were taken from the heart, lymph nodes, liver, and leg ulcer. Pathology tissue specimens from autopsy were sent to the Infectious Diseases Pathology Branch at the Centers for Disease Control and Prevention for unexplained death evaluation. Immunohistochemical (IHC) assays for spotted fever group *Rickettsia* species and HRTV were performed by using a polymer-based indirect immunalkaline phosphatase detection system with colorimetric detection of antibody-polymer complex with Fast Red Chromogen (Biocare Medical, Concord, California). RNA was extracted from FFPE liver, gallbladder, spleen, and pancreas autopsy tissues and evaluated by a conventional reverse transcription polymerase chain reaction (RT-PCR) assay [6].

## RESULTS

Significant findings on external gross examination include left lower extremity erythema with a 2-cm area of central necrosis on the lateral calf. Internal gross exam revealed geographic necrosis of the liver and mottling or softening of the splenic parenchyma consistent with infarction. The only observed manifestations of chronic disease included a 1.2-cm remote infarct in the right thalamus corresponding to the area of a prior stroke, arterio- and arteriolonephrosclerosis of the kidneys, left ventricular hypertrophy, and mild atherosclerotic heart disease.

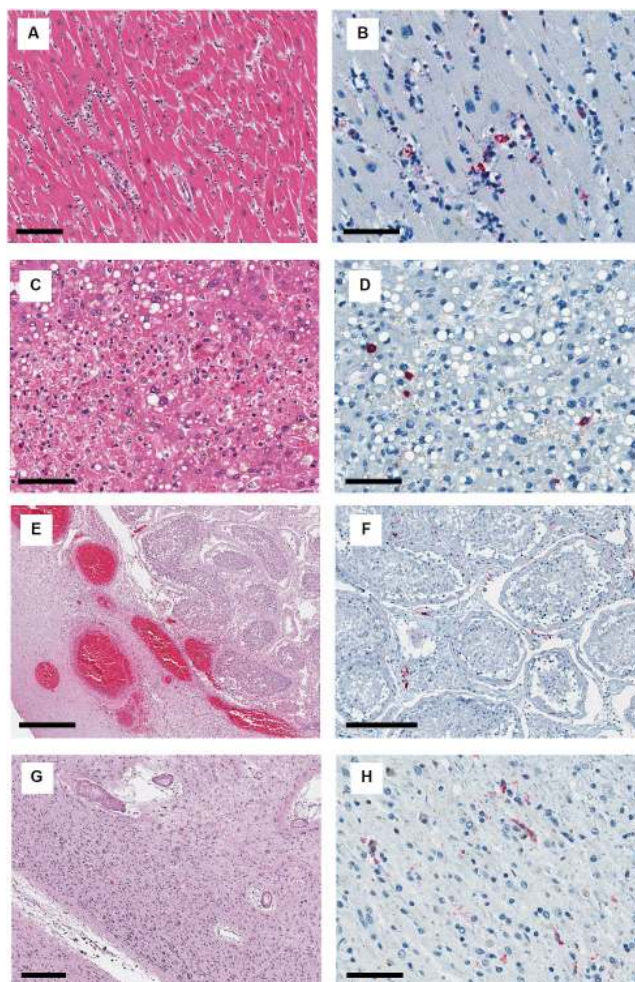
IHC assays for spotted fever group *Rickettsia* were negative; however, HRTV IHC was positive in the brain (thalamus), liver, pancreas, heart, lung, large bowel, small bowel, kidney, testes, bone marrow, lymph nodes, spleen, and muscle (left calf) (Figure 1, A–H). Evidence of lymphocytic myocarditis with viral antigens was present in the inflammatory foci (Figure 1A and 1B). In the liver, viral antigens were present in macrophages and Kupffer cells amid a background of steatosis, focal hepatocellular necrosis, and Kupffer cell hyperplasia (Figure 1C and 1D). The kidneys demonstrated hemorrhage, interstitial inflammation, scattered glomerulosclerosis, and acute tubular necrosis with viral antigens present within scattered glomeruli. A splenic infarction and hemorrhage with abundant viral antigens in the infarct area was present. The testis demonstrated congestion and focal hemorrhage with viral antigens predominantly in the interstitial and connective tissue fibroblasts (Figure 1E and 1F), but rare immunostaining was also observed in the seminiferous tubules. Occasional hemophagocytosis was found in the bone marrow, with granular immunostaining of viral antigens in mononuclear cells. The thalamus, the area of a prior cerebral infarct, demonstrated gliosis, edema, and necrosis with viral antigens present within glial cells (Figure 1G and 1H). HRTV RNA was also identified in liver, gallbladder, pancreas, and spleen by RT-PCR and sequencing.

## DISCUSSION

HRTV can cause rapidly fatal, widely disseminated infection with severe shock and multisystem organ failure in patients without substantial comorbidities. Although this patient did have past comorbidities, his only active medical condition was hypertension, and autopsy demonstrated limited manifestations of chronic disease. This differs from the previously reported HRTV-attributed fatality, a patient aged 80 years with a history of deep vein thrombosis, chronic obstructive pulmonary disease, and heavy alcohol use with multiple manifestations of chronic disease evident at autopsy [5]. The 2 patients who died from HRTV infections did have multiple similar laboratory findings, including thrombocytopenia, hyponatremia, elevated liver function studies, elevated creatine kinase, and elevated lactate dehydrogenase (LDH) levels, although certain laboratory abnormalities were more severe in this patient [5]. Of note, studies in China have identified older age, decreased level of consciousness, elevated LDH levels (>1200 IU/L), and elevated levels of creatine kinase (>800 U/L) as significant predictors of death in patients with SFTS [7].

Despite similarities, this case is distinct from previously reported HRTV cases. First, this patient never developed leukopenia or neutropenia, but had significant leukocytosis (peak of  $31.1 \times 10^3$  cells/ $\mu$ L) with left shift (white blood cell differential range, 67%–99% neutrophils). Furthermore, this patient demonstrated extreme hyperferritinemia (range, 32662–46789 ng/mL; reference range, 24–336 ng/mL), which has not been documented in any prior patient. A recent case report from Japan describes a fatal case of SFTS complicated by hemophagocytic lymphohistiocytosis; however, the patient did not meet the clinical diagnostic criteria [8, 9]. Last, novel pathologic findings were documented in this case. Viral antigen had previously only been identified in premortem bone marrow, whole blood and serum, and postmortem lymph node (mediastinal and mesenteric) and splenic tissue, whereas testing of bone marrow, liver, kidneys, adrenal glands, and central nervous system specimens had previously been negative [1, 5]. However, in this patient, viral antigen was widely distributed in multiple tissues, including brain (thalamus), liver, gallbladder, pancreas, heart, lung, large and small bowel, kidney, and testes, in addition to the previously described bone marrow, lymph node, and splenic tissue. The presence of HRTV antigen in the brain infarct correlates with the clinical presentation of altered mental status and delirium, and HRTV in the large and small bowel might explain the frequency of gastrointestinal symptoms reported early in disease courses for multiple patients. This patient also developed severe metabolic acidosis and renal failure, with HRTV antigen identified within scattered glomeruli. The presence of HRTV antigen in the testes is of undetermined significance and raises the possibility that HRTV might be sexually transmitted. Novel routes of transmission are a growing area of research because other viruses, such as Zika and Ebola, have recently been determined to spread through sexual transmission [10, 11].

Although no current treatment or routine testing is available for HRTV, there are important public health implications from



**Figure 1.** Novel histopathologic findings and immunolocalization of viral antigens in Heartland virus (HRTV)-attributed fatality. *A*, Heart: focus of lymphocytic myocarditis. *B*, HRTV antigen detected in myocardium. *C*, Liver: steatosis, focal hepatocellular necrosis, and Kupffer cell hyperplasia. *D*, HRTV antigen detected in macrophages and Kupffer cells in liver. *E*, Testis: congestion and focal hemorrhage. *F*, HRTV antigen detected in interstitial and connective tissue fibroblasts and, rarely, in seminiferous tubules in testis. *G*, Brain, thalamus: gliosis, edema, and necrosis. *H*, HRTV antigen detected in glial cells in brain. Routine hematoxylin and eosin stain: *A*, *C*, *E*, *G*. Immunoalkaline phosphate staining, naphthol fast red substrate with light hematoxylin counterstain: *B*, *D*, *F*, *H*. Magnification (scale bar measurements): *A*, 100  $\mu\text{m}$ ; *B*–*D*, 60  $\mu\text{m}$ ; *E*, 300  $\mu\text{m}$ ; *F* and *G*, 200  $\mu\text{m}$ ; *H*, 60  $\mu\text{m}$ .

additional cases. Healthcare providers should contact their local or state health department if they have a patient with an acute illness compatible with HRTV.

In conclusion, HRTV can cause rapidly fatal, widely disseminated infection in patients without substantial preexisting comorbidities. We identified viral antigen in multiple organ tissues where it was not detected previously.

## Notes

**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.** All authors: No potential conflicts.

All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

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