

Unwinding the mystery of Heartland virus disease

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July 2014—Missouri is no country for old men. Clinical, laboratory, and epidemiologic investigations conducted during the past five years have identified a new virus that can cause considerable illness and perhaps even death. All eight patients definitively proven to be ill with this virus have been over age 50; seven lived in Missouri.

However, it's unlikely that infection with the new virus, called Heartland virus, or HLV, is limited to men over 50 who live in Missouri. (For that matter, not everyone would agree that being over age 50 is old.) Rather, the demographics of HLV infection to date reflect the age and occupations of the region's male population. "Some of these men [who were infected with HLV] were farmers and all spent a lot of time outdoors," Scott Folk, MD, who cared for the first two patients in whom HLV was identified, tells CAP TODAY. Current evidence suggests that HLV is spread by the bite of infected Lone Star ticks (*Amblyomma americanum*).



Dr. Folk

"In Northwest Missouri tick bites are a way of life for farmers," says Dr. Folk, who is a specialist in adult infectious diseases at the Heartland Clinic in St. Joseph, Mo. "Because they work outdoors, they are exposed to tick bites at the time of the year when ticks, especially nymphs, are out"—primarily spring and early summer.

There is no biological reason why HLV infection should be restricted to older men. "It has to do with risk of exposure," Dr. Folk continues. "There is nothing to prevent a young man or young woman from getting a tick bite and acquiring infection."

That farmers are at increased risk is "definitely one theory and potentially a very good one," says J. Erin Staples, MD, PhD, leader of the field studies on the virus and a medical epidemiologist in the Arboviral Diseases Branch of the Centers for Disease Control and Prevention in Fort Collins, Colo.

As for why the first victims of HLV were found in Northwest Missouri, one could argue it is because Dr. Folk practices there. During the 1990s, after an infectious disease fellowship at Mayo Clinic, Dr. Folk worked in Tallahassee, Fla., where he saw patients with symptoms of human monocytic ehrlichiosis, or HME—high fever, chills, headaches, and low white blood cell and platelet counts. During his time at Mayo, Dr. Folk had become familiar with HME, which is caused by the obligate intracellular bacterium *Ehrlichia chaffeensis*, also transmitted by the bite of the Lone Star tick. While in Tallahassee, he sent samples from patients whom he considered likely candidates for HME to William Nicholson, PhD, of the CDC to identify *E. chaffeensis*. "In 1994 how did you prove the diagnosis of HME? In those early days there was only PCR," Dr. Folk says. "I sent specimens to CDC, and some were positive."

When he moved to Heartland Regional Medical Center in 1998, Dr. Folk continued to send suspicious specimens to Dr. Nicholson. In a talk on the discovery of HLV at the 2014 Clinical Virology Symposium, Dr. Folk shared that one specimen came from a 57-year-old man with HME-like symptoms whom he saw in June 2009. A few weeks later he sent samples from a 67-year-old man. "Both were farmers from Northwest Missouri and both had fever, fatigue,

diarrhea, thrombocytopenia, and leukopenia,” Dr. Folk says. “And both had been bitten by ticks five to seven days before the onset of illness.” The second patient had had 50 to 60 tick bites during one day. Neither man had a localized rash.

While awaiting results from the CDC, Dr. Folk put both patients on doxycycline, the recommended treatment for HME. Here is where he got the first indication that he had encountered something different. “In my experience most patients with Ehrlichia feel better and start to demonstrate laboratory improvement within two to three days following initiation of doxycycline therapy,” he says. “Their fever starts to resolve.” These two patients did not improve. Cytopenia and fever continued, liver function tests worsened, and laboratory abnormalities persisted. Clinical improvement was slow.

In the second patient things were so bad that a bone marrow biopsy was ordered to rule out a hematologic abnormality, such as myelodysplastic syndrome. The two patients stayed in the hospital for 10 and 12 days. One took four to six weeks to make a full recovery. “This was unusual in my experience,” Dr. Folk says.

Meanwhile, in the CDC laboratories, samples from both of these patients were negative for *E. chaffeensis*. Also, cytopathic changes were seen in inoculated cells. Electron microscopy visualized particles consistent with the bunyavirus family. According to the published report on the identification of HLV, “Next-generation sequencing and phylogenetic analysis identified the viruses as novel members of the phlebovirus genus” (McMullan LK, et al. *N Engl J Med.* 2012; 367:834-841). “There was 95 percent to 99 percent identity among the three viral genetic segments between the two patients,” Dr. Folk says. It was named Heartland virus after the medical center in which the first two patients were seen.

In his symposium talk, Dr. Folk pointed out that this was the first new human virus infection identified in the United States since hantavirus in 1993.

In an interview, the CDC’s Dr. Staples, who was not involved in identifying HLV, noted that the sequence of the new bunyavirus was so novel that the investigators “almost didn’t believe it.” Fortunately, the Chinese Center for Disease Control and Prevention produced an article in 2011 about a novel phlebovirus in 171 Chinese patients that caused “severe fever with thrombocytopenia syndrome” (Yu XJ, et al. *N Engl J Med.* 2011;364:1523-1532). When the sequence of the novel U.S. virus was compared with that of the Chinese phlebovirus, there was about 70 percent similarity.

Dr. Staples entered the HLV story in 2011. “Phleboviruses are transmitted to people through the bite of arthropods—mosquitoes, ticks, and sand flies,” she says. Lone Star ticks from Missouri were found to be positive for HLV by RT-PCR (Savage HM, et al. *Am J Trop Med Hyg.* 2013;89:445-452). Since Dr. Staples works in the Arboviral (arthropod-borne viruses) Diseases Branch, she was called in for the next step, which was to set up studies to systematically identify new patients, find out how they were getting infected, and identify risk factors or groups at higher risk.

Dr. Staples coordinated a study in northwestern Missouri with the Missouri Department of Health and Senior Services, with seven medical institutions participating. Starting in early 2012, they prospectively enrolled people who matched the clinical case definition: fever of 100.4 degrees or higher, white blood cell count less than 4.5×10^3 , and platelet count less than 150,000. “Could we be missing some infected people with this case definition? “Absolutely,” Dr. Staples says, adding, “Unfortunately, this disease is relatively nonspecific. We can expand it later to catch more infected patients.” What was needed at this early stage was a diagnostic test. “We needed to know definite Heartland virus infection to validate our assay. We now have RT-PCR for viral RNA detection, ELISA for IgG antibodies, and a plaque reduction neutralization test for detecting neutralizing antibodies,” she says.

“What we are still missing, and why we are still doing this survey prospectively, even this year, is a reliable IgM assay,” Dr. Staples explains. “That would be important to diagnose people who are more acutely infected.”

So far the study has identified six additional people infected with HLV, four of whom were hospitalized, like Dr. Folk's original two patients (Pastula DM, et al. *Morb Mortal Wkly Rep.* 2014;63:270-271). Those six subjects represent about 10 percent of the patients included in the study, Dr. Staples says. "From the cases we've identified so far it looks very similar to disease caused by Ehrlichia. However, as Dr. Folk observed, patients [infected with HLV] don't respond to the usual Ehrlichia treatment of doxycycline within 48 hours."

According to Dr. Staples, what remains to be shown to verify that HLV is transmitted by Lone Star ticks is to demonstrate that when infected ticks bite they regurgitate the virus. "Experiments are going on right now in our lab to prove that," she says.

"Since we do believe that this Heartland virus will be transmitted to people by the bite of a mosquito or tick," Dr. Staples continues, "they can protect themselves by being aware of ticks. If they remove ticks before they become infected, it might help prevent them from becoming unwell."

In addition to the study in Missouri, the CDC is conducting one across the U.S. in which physicians can submit samples from patients from other states. "We don't know how prevalent this infection is going to be," Dr. Staples says. To address this question, they will look eventually for the presence of anti-HLV antibodies. There could also be a difference in the clinical presentation between men and women. "That would be a bit unusual," she says. "Although with West Nile virus, older men are more likely to get encephalitis than women."

Another important goal is to establish the range of HLV. Is it co-extensive with the range of the Lone Star tick, which extends from Maine to Florida and Texas? "That is still an unanswered question," Dr. Folk says. "Can infected ticks be found in other parts of that range besides Missouri?" □

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