Heartland virus (HRTV; Phenuviridae, Bandavirus) is an emerging human pathogen initially isolated from patients in Missouri, USA, during 2009 (1). Since then, >50 known human cases have been identified in Arkansas, Georgia, Illinois, Indiana, Iowa, Kansas, Kentucky, Missouri, North Carolina, Oklahoma, and Tennessee (2–5). Amblyomma americanum, the lone star tick, has been implicated in HRTV transmission and maintenance (6–8). Small-sized and medium-sized mammals and ground dwelling birds, such as wild turkeys (Meleagris gallopavo), serve as hosts for immature ticks. Adult ticks feed primarily on large mammals, such as coyotes (Canis latrans) and white-tailed deer (Odocoileus virginianus). Ticks at all 3 active developmental stages will bite humans (9). Serologic evidence in mammal hosts, including white-tailed deer, indicates that HRTV is distributed primarily in the Midwest and southeast United States, as well as the northeastern Atlantic coast (10–12).

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During 2018, Heartland virus RNA was detected in an Amblyomma americanum tick removed from a resident of Suffolk County, New York, USA. The person showed seroconversion. Tick surveillance and white-tailed deer (Odocoileus virginianus) serosurveys showed widespread distribution in Suffolk County, emphasizing a need for disease surveillance anywhere A. americanum ticks are established or emerging.

Heartland Virus Transmission, Suffolk County, New York, USA
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During August 2018, New York State Department of Health (NYSDOH) epidemiologists were notified that HRTV RNA was detected in an A. americanum nymph removed from a resident of Long Island, New York, USA. This infected tick was tested at the University of Massachusetts (https://www.tickreport.com).

In response, the NYSDOH and Suffolk County Department of Health Services conducted tick surveillance and performed HRTV serologic analysis on the person from whom the tick was removed. Analysis was also performed for a hunter-harvested white-tailed deer in Suffolk County.

The Study
Officials with the NYSDOH and Suffolk County Department of Health Services contacted a Long Island, New York, resident for a follow-up investigation after receiving notification that a tick removed from the resident and submitted for comprehensive pathogen testing was positive for HRTV RNA. The resident, a man in his 60s, removed the tick on August 8, 2018, and recalled having a low-grade fever (maximum temperature 100.5°F) and fatigue for 5 days beginning on August 15, 2018. He noted no other symptoms.

Serum was provided at multiple time points for serologic analysis. We tested serum samples by using a standard 90% plaque reduction neutralization test (PRNT90) for HRTV (strain M12-66) (8), provided by the Centers for Disease Control and Prevention. We tested samples at Wadsworth Center, NYSDOH, and results were confirmed by the Centers for Disease Control and Prevention. Neutralizing antibody titers were 1:20, 1:160, and 1:160 for samples collected at 8, 50, and 96 days after symptom onset (15, 57, and 103 days after removal of the tick), respectively, indicative of a recent infection with HRTV.

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Heartland Virus Transmission, New York, USA

We initiated standardized drag and flag sampling of host-seeking *A. americanum* ticks on public lands for arbovirus surveillance during 2016, before HRTV detection. We found that 132 pools (containing 475 nymphs and 437 adults) from 4 Suffolk County locations were negative for HRTV by real-time reverse transcription PCR using established protocols (8). During 2018, tick surveillance at 5 locations yielded 102 pools (969 adults); all were negative for HRTV.

Increased efforts during the public health investigation conducted on August 23 and 24, 2018, yielded an additional 113 *A. americanum* ticks (92 larvae and 21 nymphs) from a location where tick exposure potentially occurred. All ticks collected during the investigation were negative for HRTV. No ticks were found during sampling of the property surrounding the residence of the case-patient.

During 2019 and 2020, tick surveillance in the towns of Brookhaven and Riverhead yielded 1,123 pools of *A. americanum* ticks (2,788 adults and 6,728 nymphs) (Figure 1). We found that 3 pools of unengorged nymphs collected from the Brookhaven site on June 14 (n = 1) and June 24 (n = 2), 2019, and 2 pools of unengorged nymphs collected from the same location on July 25 and August 5, 2020, were positive for HRTV RNA. We isolated virus from 2 tick pools after incubation on Vero cells. We found that testing of >1,100 *Ixodes scapularis* ticks (199 pools) collected during the surveillance campaign in Suffolk County, during 2018–2020, were negative for HRTV.

We extracted RNA from isolates by using established protocols (13). We developed primer pairs to amplify the small, medium, and large RNA segments by using a One-Step Superscript III Reverse Transcription PCR with Platinum Taq (Life Technologies, https://www.thermofisher.com) (Table 1). We performed 3 separate reactions using 5 µL of RNA, 1 µL of polymerase, and 0.2 µmol/L final concentration of primer pairs in a total reaction volume of 50 µL. We amplified products with the following thermocycler

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**Figure 1.** Tick collection sites in study of heartland virus transmission, Suffolk County, New York, USA. Numbers within townships indicate sample size of deer tested for neutralizing antibody.

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Table 1. Primer pairs for amplifying small, medium, and large RNA segments of Heartland virus genome

| Name        | Sequence, 5′→3′          | Target     |
|-------------|--------------------------|------------|
| HRTV_S_F    | TTACACAAAGAACCCTTGAAATTACA | Small      |
| HRTV_S_R    | CATCAAGCATGACTGCTGGTCTGCAAAT | Small      |
| HRTV_M_F    | AAGTAGGGTAAACCGCTATCCACCTGAGAT | Medium    |
| HRTV_M_R    | ACAAAGACCGCTATACAAATGAAAC | Medium    |
| HRTV_L1_F   | GACGTCCAGATGAATTTAGAAGCTTCTT | Large     |
| HRTV_L1_R   | CATAGCTGCTTGGTATGTTCTGCT | Large     |
| HRTV_L2_F   | TGCAAGAGATGATGACACCTC     | Large     |
| HRTV_L2_R   | AAGACCTGCTAGATCCCACCTTAGG | Large     |

Table 2. Pairwise genetic distance (% of variable positions) among fully sequenced isolates of Heartland virus, by segment (small/medium/large), for nucleotide (below diagonal) and amino acid (above diagonal) sequences

| Isolate (reference) | NY20-1782 | NY20-1820 | MO 2009-P1 | MO 2009-P2 | TN 2013 |
|---------------------|-----------|-----------|------------|------------|---------|
| NY20-1782, MZ440344/MZ440346 | 0/0/0 | 0.54/0.46/0.43 | 0.72/1.67/0.34 | 1.26/0.46/0.19 |
| NY20-1820, MZ440341/MZ440343 | 0/0.03/0.61 | 0.54/0.46/0.43 | 0.72/0.43/0.34 | 1.26/0.46/0.19 |
| MZ440342* | 1.41/1.43/1.84 | 1.41/1.46/2.45 | 0.54/1.77/0.77 | 1.26/0.37/0.62 |
| MO 2009-P1 (1), JX005842.1/JX005844.1/JX005846.1* | 1.69/4.32/1.88 | 1.69/4.35/2.49 | 1.45/4.52/1.90 | 1.44/0.77/0.53 |
| MO 2009-P2 (1), JX005843.1/JX005845.1/JX005847.1* | 2.34/2.45/1.98 | 2.34/2.48/2.50 | 2.06/2.22/2.00 | 2.57/4.91/1.95 |
| TN 2013 (2), KJ740146.1/KJ740147.1/KJ740148.1* | 3.17/3.15/2.39 | 3.17/3.15/2.39 | 2.94/2.92/2.00 | 2.57/4.91/1.95 |

*GenBank accession numbers.
Results from this study emphasize the need to include HRTV in surveillance programs wherever *A. americanum* ticks are distributed. Furthermore, clinicians should be aware of this pathogen and the potential for overlapping symptomologies (fever, fatigue, and loss of appetite) with other tickborne infections. Providers should request HRTV testing for patients who have clinical symptoms, including leukopenia and thrombocytopenia, and a history of tick exposure or travel to regions where *A. americanum* ticks are reported.

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About the Author

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Since the 2015 Zika virus outbreak in the Americas, transmission of this vectorborne disease has substantially decreased. But Zika virus doesn’t spread only through mosquito bites...it also spreads through sexual transmission, blood transfusions, breastfeeding, and even needlestick injuries in laboratories.

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