Heartland virus (HRTV), a phlebovirus in the order Bunyavirales, is an emerging zoonotic pathogen. In 2009, after 2 cases were identified in persons in Missouri, additional cases were subsequently reported from Kansas, Oklahoma, Arkansas, Missouri, Tennessee, Kentucky, Indiana, Georgia, and South Carolina. Disease onset was most often during April–September (1). HRTV symptoms can initially resemble those of ehrlichiosis (2) and include fatigue, fever, leukopenia, and thrombocytopenia (3). Human illness caused by HRTV infection often requires hospitalization and has resulted in death (1).

After 2 persons infected with HRTV in northwestern Missouri reported having noticed attached ticks before symptom onset (4), subsequent entomologic studies detected HRTV in nymphal *Amblyomma americanum* ticks. Laboratory studies confirmed the competence of *A. americanum* ticks for transmitting HRTV transstadially and horizontally (5). This body of evidence led to the implication of *A. americanum* ticks as the putative vector of HRTV (2,6). Serologic surveys of mammals and birds subsequently detected HRTV-specific neutralizing antibodies in a variety of mammals, including raccoons and white-tailed deer, suggesting that various medium- and large-sized mammals may serve as hosts (3,7).

*A. americanum* ticks are vectors of public health concern because of their aggressive biting behavior, willingness to feed on humans, and abundance. Over the past century, their distribution range has expanded northward (8), and population establishment continues to increase because of climate change (9). Habitat suitability models have suggested that this species’ fundamental niche should reach the center of Illinois (10) or eventually encompass the state entirely (9).

In July 2018, a Kankakee County, Illinois, resident (case-patient 1) reported having incurred multiple tick bites while camping on private residential property. The patient was hospitalized with fever, headache, myalgia, nausea, diarrhea, and a diffuse maculopapular rash. In September 2018, a Williamson County, Illinois, resident (case-patient 2) noticed tick bites while staying at a campground near home. The patient was hospitalized with fever, headache, myalgia, fatigue, decreased appetite, nausea, and diarrhea. The Centers for Disease Control and Prevention (CDC) confirmed that clinical samples from both patients were positive for HRTV. We subsequently performed entomologic investigations to determine tick density and HRTV prevalence among tick populations at the likely sites of exposure.

The Study
The suspected sites of human exposure were determined according to case-patient interviews conducted by local county health departments (Figure). Two of the 3 sites were in an area considered endemic for *A. americanum* ticks, and the other site was near the putative current northern distribution range limit for this tick vector.
For case-patient 1, the potential exposure site was an ≈40-acre rural homestead in Kankakee County, which had an assemblage of barnyard animals, including chickens, goats, horses, and turkeys (site 1) and a small amount of forest surrounded by extensive cropland. For case-patient 2, in Williamson County, a potential exposure site consisted of 2 adjacent lakeshore campgrounds located within a heavily wooded wildlife refuge (site 2) and another was a suburban home with sparse tree cover (site 3). We observed deer at site 1 during collection visits on June 21 and 25, 2019, and deer, coyotes, and raccoons at site 2 during visits on July 11 and 12, 2019. A pet dog lived at the residence at site 3, which we visited on July 11, 2019.

We collected ticks by dragging along 150-m transects (sites 1 and 2) and with carbon dioxide traps consisting of a 1 m² white cloth laid on the ground with 0.5 kg of dry ice left in the center to sublimate for 2 hours before returning to collect ticks (sites 1–3). We collected live ticks into 14-mL plastic centrifuge tubes (TPP, https://www.tpp.ch) that had been

Figure. Tick collection sites associated with 2 cases of Heartland virus infection in humans, Kankakee and Williamson Counties, Illinois, USA, 2019. Locations of the counties are indicated by red dots on the Illinois map.
modified by applying carpet tape between the lid and tube mouth. We added ticks through a tape-covered hole punched in the center of the paper-backed side of the tape; the sticky side of the tape facing the tube interior immobilized the ticks before they could exit, enabling their secure transport while alive (Video, https://wwwnc.cdc.gov/EID/article/26/7/20-0110-V1.htm). Ticks were either kept alive (site 1) or killed in the field at the end of the day and kept on dry ice (sites 2 and 3) during transport to the Illinois Natural History Survey Medical Entomology Laboratory (Champaign, IL, USA), where they were identified and sorted by species, life stage, and sex (11,12) on a chill table and maintained at -80°C. Ticks were then shipped on dry ice to the CDC Arboviral Diseases Branch (Fort Collins, CO, USA) for Heartland and Bourbon virus testing, where tick pool homogenization, RNA extraction, and virus screening were performed by real-time PCR as previously described (2,13). The prevalence of virus infection from pooled samples was calculated by using PooledInfRate, which implements a bias-corrected maximum-likelihood estimation method (14).

A total of 70 pools of adult ticks and 23 pools of nymphs were tested (Table 1). The median pool size for adult ticks was 10 (range 1–10) and for nymphs was 30 (range 3–33). A single pool of male *A. americanum* ticks from each county was positive for HRTV (cycle threshold values of 21.7 for site 1 and 24.1 for site 2 by first PCR, 23.2 and 25.3 after confirmation by second PCR); Bourbon virus was not detected. The estimated prevalence of HRTV in adult male *A. americanum* ticks was 9.46/1,000 ticks at site 1 and 7.60/1,000 ticks at site 2 (Table 2).

**Table 1. Collection methods and number of ticks of each species and life stage collected in 2 counties, Illinois, USA, 2019**

| Site, method, tick species | Stage | Sex | No. collected | Density/1,000 m² |
|----------------------------|-------|-----|---------------|-----------------|
| **Site 1**†                |       |     | 659           |                 |
| Dragging                   |       |     |               |                 |
| *Amblyomma americanum*     | Adult | F   | 93            | 26              |
|                           | Adult | M   | 90            | 25              |
|                           | Nymph | Not applicable | 338            | 93              |
| *Dermacentor variabilis*   | Adult | F   | 15            | 4               |
|                           | Adult | M   | 10            | 3               |
| Carbon-dioxide trap        |       |     |               |                 |
| *A. americanum*            | Adult | F   | 18            | Not applicable  |
|                           | Adult | M   | 17            | Not applicable  |
|                           | Nymph | Not applicable | 75             | Not applicable  |
| *D. variabilis*            | Adult | F   | 1             | Not applicable  |
|                           | Adult | M   | 1             | Not applicable  |
| *Ixodes scapularis*        | Nymph | Not applicable | 1              | Not applicable  |
| **Site 2†**                |       |     | 498           |                 |
| Dragging                   |       |     |               |                 |
| *A. americanum*            | Adult | F   | 32            | 15              |
|                           | Adult | M   | 44            | 21              |
|                           | Nymph | Not applicable | 159            | 76              |
| *D. variabilis*            | Adult | F   | 1             | 0.5             |
|                           | Adult | M   | 2             | 1               |
| Carbon-dioxide trap        |       |     |               |                 |
| *A. americanum*            | Adult | F   | 118           | Not applicable  |
|                           | Adult | M   | 88            | Not applicable  |
|                           | Nymph | Not applicable | 48             | Not applicable  |
| *D. variabilis*            | Adult | F   | 3             | Not applicable  |
|                           | Adult | M   | 3             | Not applicable  |
| **Site 3‡**                |       |     | 9             |                 |
| Carbon-dioxide trap        |       |     |               |                 |
| *A. americanum*            | Adult | F   | 4             | Not applicable  |
|                           | Nymph | Not applicable | 4              | Not applicable  |
| *D. variabilis*            | Adult | F   | 1             | Not applicable  |

*Site 1, Kankakee County, visited June 21 and 25, 2019; dragging, n = 24 × 150 m transects; carbon dioxide traps, n = 3.
†Site 2, Williamson County, visited July 11–12, 2019; dragging, n = 14 × 150 m transects; carbon dioxide traps, n = 9.
‡Site 3, Williamson County, visited July 11–12, 2019; no dragging performed because of site size; carbon dioxide trap, n = 2.

**Conclusions**

One year after 2 cases in humans were detected, HRTV was detected in *A. americanum* ticks collected from the suspected exposure locations in Illinois. Because of abundant suitable habitat and established *A. americanum* tick populations (10), it is notable and predictable that this pathogen emerged in southern Illinois. The density of and HRTV detection in *A. americanum* ticks at the northern edge of their distribution range in Kankakee County was unexpected. Our findings suggest that *A. americanum*
Table 2. Prevalence of Heartland virus in ticks, by location, species, and sex in 2 counties in Illinois, USA, 2019*

| Species                  | Stage | Sex | County    | No. ticks collected | No. pools | No. positive pools | Infection rate/1,000 ticks, MLE (95% CI) |
|--------------------------|-------|-----|-----------|---------------------|----------|-------------------|----------------------------------------|
| *Amblyomma americanum*   | Adult | M   | Kankakee  | 107                 | 16       | 1                 | 9.46 (0.55–46.1)                       |
| *A. americanum*          | Adult | F   | Kankakee  | 111                 | 12       | 0                 | 0 (0–29.5)                            |
| *A. americanum*          | Nymph | NA  | Kankakee  | 413                 | 15       | 0                 | 0 (0–8.2)                             |
| *A. americanum*          | Adult | M   | Williamson| 132                 | 15       | 1                 | 7.6 (0.44–36.9)                       |
| *A. americanum*          | Adult | F   | Williamson| 154                 | 17       | 0                 | 0 (0–22.16)                          |
| *A. americanum*          | Nymph | NA  | Williamson| 211                 | 8        | 0                 | 0 (0–14.5)                           |
| *Dermacentor variabilis* | Adult | Both | Kankakee  | 27 (16 F, 11 M)     | 4        | 0                 | 0 (0–92.8)                           |
| *D. variabilis*          | Adult | Both | Williamson| 10 (5 F, 5 M)       | 6        | 0                 | 0 (0–248.8)                          |

*MLE, maximum-likelihood estimation; NA, not applicable.

tick collections and wildlife serosurveys will help assess whether transmission cycles are active in Illinois and enhance our knowledge of the transmission ecology of this rare pathogen.

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