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Heartland Virus Exposure in White-Tailed Deer in the Southeastern United States, 2001–2015

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Abstract. Heartland virus (HRTV) is a North American *phlebovirus* suspected to be transmitted by the lone star tick *Amblyomma americanum*. White-tailed deer (WTD) have been shown to develop HRTV-neutralizing antibodies following experimental infection. To further define the geographic distribution of HRTV through retrospective sampling of WTD, sera from the WTD herd health serum archive at the Southeastern Cooperative Wildlife Disease Study between 2001 and 2015 were analyzed using serum neutralization. Of 783 serum samples tested, 57 (7.3%) were positive for HRTV-neutralizing antibodies. Deer with moderate to heavy tick burdens were more likely seropositive. Seropositive samples were obtained from deer originating from states with documented human cases of HRTV-associated disease. Seropositive samples were identified from years before the recognition of the first human case in 2009. Overall, this study indicates that WTD in the southeastern United States have been exposed to HRTV as early as 2001 and that the presence of seropositive animals corresponds roughly with reported human HRTV-associated disease.

Heartland virus (HRTV; family Phenuiviridae, genus *Phlebovirus*) was first recognized in 2009 as a cause of febrile disease with thrombocytopenia and leukopenia that has since been documented in 30 people, including at least one fatal case. Human disease has been reported in nine states, mainly in the Midwestern and southern United States.[1–4] The virus is most likely transmitted by the lone star tick *Amblyomma americanum* and has been isolated from wild-caught ticks in the vicinity of the first reported human cases,[5,6] as well as experimentally transmitted by co-feeding between *A. americanum* ticks.[7] *Amblyomma americanum* has a vast and expanding geographical range including 39 states and the District of Columbia, predominantly in the southeastern states (as far west as Texas), and extending north through the mid-Atlantic and New England regions.[8]

Heartland virus–reactive antibodies have been detected in serum samples of free-ranging white-tailed deer (WTD) (*Odocoileus virginianus*), in the central and eastern United States.[9,10] White-tailed deer have recently been experimentally infected, and although they did not become viremic or shed virus, they did develop neutralizing antibody titers or increases in existing titers.[11] White-tailed deer are also known to be a host for *A. americanum*[12] and could act as an important host for maintaining the tick vector where virus can be transmitted between arthropods by co-feeding.

Although current evidence indicates that HRTV is likely maintained within the tick population without involvement of a mammalian host,[7] seropositive animals may be useful as sentinel indicators of the presence of HRTV in an area.[9,10] The Southeastern Cooperative Wildlife Disease Study (University of Georgia) has maintained an archive of free-ranging WTD serum collected during herd health evaluations from approximately 150 sampling sites throughout the southeastern United States. While previous retrospective serosurveys for HRTV-reactive antibodies have investigated serum samples from as far back as 2009,[9] the present study evaluates older archived samples from 2001 to 2015. The objectives of the present study were to 1) determine whether there are HRTV-seropositive WTD in the southeastern United States, 2) determine when and where neutralizing antibodies first appeared relative to the recognition of human disease, and 3) associate the presence of neutralizing antibodies to the presence of ticks.

Between 1 and 10 individual WTD serum samples were available from each sampling location and year, which was recorded by county or parish, state, and date. Serum samples were thawed and diluted 1:10 in virus media comprising Minimum Essential Medium™ (MEM; Sigma-Aldrich, Darmstadt, Germany) with 3% bovine serum albumin, and 5% antibiotics and antimycotics (virus media). The samples were then heat-inactivated at 56°C for 45 minutes, then challenged with 100 tissue culture infectious dose ($TCID_{50}$) of HRTV suspension for 1 hour at 37°C to screen at a 1:20 dilution. Wells were then seeded with Vero E6 cells and incubated at 37°C for 7 days. The samples were considered positive if there was > 50% neutralization. Samples testing positive for neutralizing antibodies at 1:20 were titrated in seven 2-fold serial dilutions, beginning with a 1:4 dilution and challenged with virus as described previously. Serum from an HRTV-experimentally infected fawn was used as a positive control. Samples with titers ≥ 16 were considered seropositive based on previous experimental infections.[11]

In an effort to determine the specificity of this assay, a subset of 15 HRTV-neutralizing antibody–positive samples and 15 negative samples were also evaluated for neutralizing antibody against MP-12, a live attenuated vaccine strain of the Rift Valley fever virus (family Phenuiviridae, genus *Phlebovirus*).[13] Serial 2-fold dilutions of selected samples were made, and each well was challenged with 100 $TCID_{50}$ mutagenesis passage (MP)-12 as described previously. None of the samples tested had evidence of MP-12–neutralizing antibodies. A previous study has evaluated the cross-reactivity of HRTV antibodies with other known phleboviruses in the United States, including Sunday Canyon virus, Rio Grande virus, and Lone Star virus, and no significant cross-reactivity was reported.[9]

A total of 783 serum samples were available for evaluation. Between 29 and 83 samples were available from each year, with an average of 52 (standard deviation [SD] = 18) (Supplemental Table 1, Figure 1). Gender, age, body condition, and tick burdens of all deer screened were compared with those of deer that had HRTV-neutralizing antibodies (Table 1).

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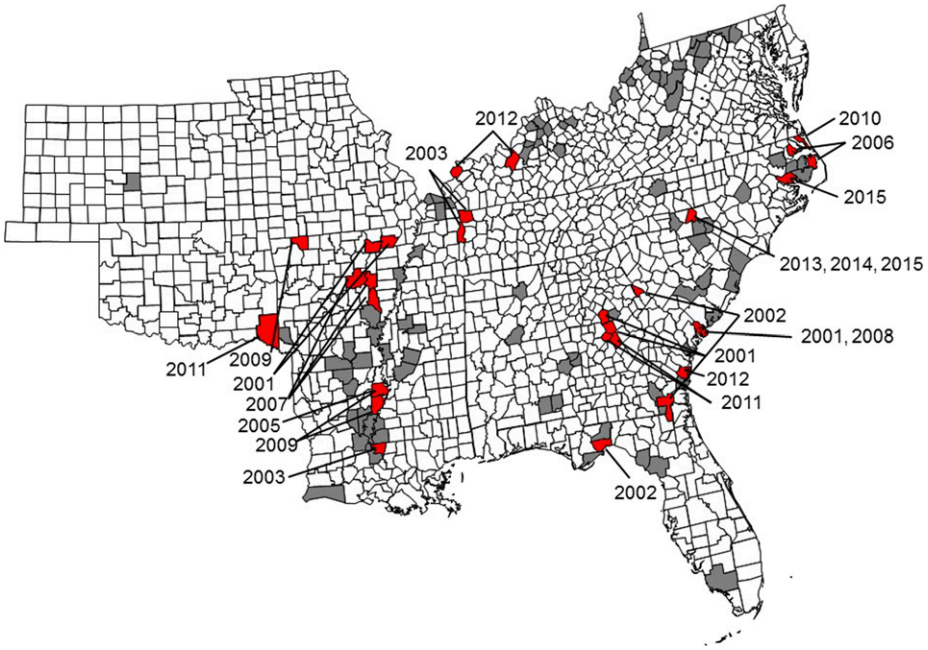

FIGURE 1. County-level distribution of where white-tailed deer serum samples were collected and where positive samples were found. Gray counties had serum samples collected but none were positive. Red counties had at least one positive sample, with the year that positive sample was collected indicated. White counties were not tested. This figure appears in color at www.ajtmh.org.

Fifty-seven (7.3%) serum samples were positive for HRTV-neutralizing antibodies (Table 2). Of all collected sera from males, 6.6% were seropositive and 7.7% of all collected sera from females were seropositive. The average age of a seropositive animal was 2.86 years (SD = 1.46), and the average age of seronegative deer was 2.70 years (SD = 1.55) (no significant difference). The 2.6–3.5 age group had the highest percentage of seropositive deer (11.6%). Of all deer with poor body condition, 10.3% were seropositive, compared with 6.7% of deer in fair condition, 6.3% of deer in good condition, and 6.9% of deer in excellent condition. Seropositive deer

TABLE 1

Summary of signalment data, body condition, and tick burdens from all deer tested via serum neutralization and deer with Heartland virus–neutralizing antibodies

	No. tested (%)	No. seropositive (%)
Total	783	57
Gender		
Male	165 (21.1)	11 (19.3)
Female	601 (76.8)	46 (80.7)
Unknown/Unreported	17 (2.1)	0 (0)
Age, year		
≤ 0.5	8 (1.0)	0 (0)
0.6–1.5	246 (31.4)	13 (22.8)
1.6–2.5	180 (23.0)	13 (22.8)
2.6–3.5	147 (18.8)	17 (29.8)
3.6–4.5	90 (11.5)	8 (14)
4.6–5.5	42 (5.4)	1 (1.8)
≥ 5.6	50 (6.4)	5 (8.8)
Unknown/Unreported	20 (2.5)	0 (0)
Body condition		
Poor	87 (11.1)	9 (15.8)
Fair	462 (59.0)	31 (54.4)
Good	159 (20.3)	10 (17.5)
Excellent	29 (3.7)	2 (3.5)
Unknown/Unreported	46 (5.9)	5 (8.8)
Tick burden		
No ticks	349 (44.6)	2 (3.5)
Light	332 (42.4)	31 (54.4)
Moderate	61 (7.8)	18 (31.6)
Heavy	19 (2.4)	6 (10.5)
Unknown/Unreported	22 (2.8)	0 (0)

TABLE 2

Counties with Heartland virus–neutralizing antibody–positive samples, including the year samples were collected and the total number of samples tested for each time point

County or parish, state	Year	No. tested	No. (%) seropositive
Greene, AR	2001	5	2 (40)
Lawrence, AR	2001	6	1 (17)
Woodruff/Monroe, AR	2007	11	2 (18)
White, AR	2007	5	1 (20)
Carroll, AR	2009	5	1 (20)
Wakulla, FL	2002	10	4 (40)
Jones/Jasper, GA	2001	5	2 (40)
Lincoln, GA	2002	5	1 (20)
Charleton, GA	2002	5	1 (20)
Bibb/Twiggs, GA	2011	5	3 (60)
McIntosh, GA	2012	6	1 (17)
Glynn, GA	2012	6	2 (33)
Union, KY	2012	6	3 (50)
Harden, KY	2012	5	2 (40)
West Feliciana, LA	2003	5	1 (20)
Madison, LA	2005	10	2 (20)
Madison/Tensas, LA	2009	10	3 (30)
Dare, NC	2006	5	3 (60)
Perquimans, NC	2006	5	1 (20)
Currituck, NC	2010	11	3 (27)
Stanly, NC	2013	10	3 (30)
	2014	3	1 (33)
	2015	5	2 (40)
Beaufort, NC	2015	5	3 (60)
McCurtain, OK	2011	5	2 (40)
Beaufort, SC	2001	5	1 (20)
	2008	5	2 (40)
Benton, TN	2003	5	2 (40)
Stewart, TN	2003	5	2 (40)

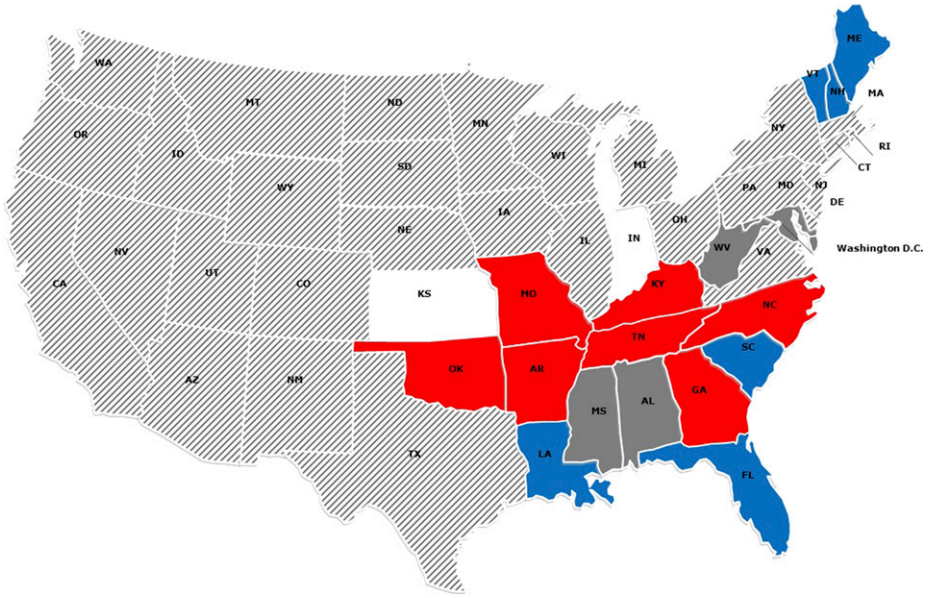

FIGURE 2. Correlation between detected Heartland virus (HRTV)–neutralizing antibody seropositive deer in the present study and reported literature[8,9] and reported human cases of human HRTV infections.[4] Red states have reported human cases of Heartland infection with seropositive deer. Blue states have no reported human cases but do have reported seropositive deer. White states have reported human cases but no reported seropositive deer. Dark gray states have no reported human cases and no reported seropositive deer. Hash-marked states have not reported human cases and have not been tested for seropositive deer. This figure appears in color at www.ajtmh.org.

were 6.25 times more likely to have a heavy tick burden than negative deer (95% confidence interval [CI]: 2.38–17.14, P = 0.0004) and 7.09 times more likely to have a moderate tick burden than negative deer (95% CI: 3.75–13.4, $P < 0.0001$). Tick species were not noted in most of the reports assessed, but given the geographic areas and the season of sampling (July through October), *A. americanum* is most likely.

Seropositive samples were collected every year except 2004. The year with the highest percentage (19.5%) and largest number (8) of positive samples collected was in 2012. Titers ranged from 16 to 128. Positive samples were from counties or parishes in nine states (Figure 1). Thirty-two positive samples were collected between 2001 and 2009. Positive counties or parishes often had clustering of 40–60% positive out of deer sampled and neighboring counties or parishes frequently had seropositive samples (Figure 1). Although the interval between sampling was variable, 33 counties were sampled in more than 1 year (Supplemental Table 2).

According to the Centers for Disease Control and Prevention,[4] human cases of HRTV infection and associated disease have occurred in Arkansas, Georgia, Indiana, Kansas, Kentucky, Missouri, North Carolina, Oklahoma, and Tennessee (Figure 2). Indiana and Missouri were not represented in our sample set, and only five samples from 1 year were available from Kansas. All other states with reported human HRTV infection had at least one seropositive animal. There were three states with seropositive deer and no currently reported human cases of HRTV infection (Louisiana, Florida, and South Carolina). A previous serosurvey similarly found that HRTV-neutralizing antibody–positive animals were present in states as of yet without human cases.[9] It is likely, and predicted,[4] that there are more cases of human exposure that go unrecognized, due in part to the nonspecific symptoms of disease in humans.

Given the sporadic geographic and temporal method of sampling and the retrospective nature of this study, there are some limitations in interpreting the dynamics of HRTV spread in the southeastern United States. There is evidence, however, of exposure to HRTV or a serologically cross-reactive virus in WTD as much as 8 years before the first recognized human case in 2009. The lack of cross-reactivity seen in the related *Phlebovirus* (MP-12) challenge, as well as previous cross-reactivity assessments,[9] suggests that serum neutralization is a specific test for HRTV. Ecological factors that affect tick populations, such as temperature, humidity, foliage types, and other mammalian hosts, may influence where WTD are likely to be exposed to HRTV, leading to clustering of positive samples. Biases may also be present in which deer were sampled because there were significantly more females than males represented and more deer over 1 year of age. More consistent sampling of WTD for HRTV-neutralizing antibodies may elucidate other factors of viral ecology and transmission and may better characterize the magnitude and duration of the humoral response in WTD.

There was a significant association with moderate and heavy tick burdens and HRTV seropositivity, which is consistent with other evidence of *A. americanum* being the primary vector for HRTV.[5–7] In addition, HRTV-exposed deer have been detected in most of the states where human cases of HRTV-associated disease have been documented. The presence of seropositive and tick-laden WTD in an area may be another useful indicator for the presence or increased circulation of HRTV in a given area, alerting human health providers and specialists to the potential of human HRTV infections.

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Note: Supplemental tables appear at www.ajtmh.org.

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