Genetic Variation and Host-Switching of New York State Heartland Virus Isolates

NEW YORK STATE | Department of Health | Wadsworth Center

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1 Background

Heartland virus (HRTV; *Phenuviridae*, *Bandavirus*) is an emerging tick-borne virus transmitted by lone star ticks (*Amblyomma americanum*). HRTV was first detected in NYS in 2018, both by isolation from *Amblyomma americanum* and seroconversion in an individual that removed the positive tick. Three additional positive tick pools were identified in Suffolk County in 2019-20 and isolates were found to be genetically distinct from HRTV from other areas. In 2021 unprecedented activity occurred, with 19 HRTV isolations from tick pools throughout the state. Another tick species distributed throughout NYS, the black-legged tick (*Ixodes scapularis*), could be of particular concern for transmission in NYS; however, HRTV has never been identified or experimentally infected in *Ixodes scapularis. Ixodes scapularis* is a competent vector for many tick-borne diseases, has a broader geographical range than *Amblyomma americanum* (see fig 1 and fig 2), and feed on similar hosts to *Amblyomma americanum*.

Fig 1. Geographical range of *Ixodes scapularis* in the United States

Fig 2. Geographical range of *Amblyomma americanum* in the United States

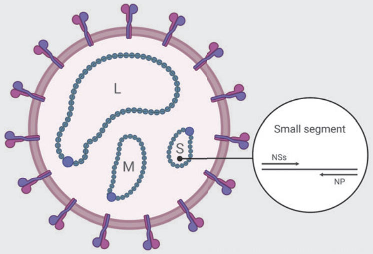


Fig 3. HRTV consisting of small, medium, and large segments (denoted by S, M, and L). Highlighted is the small segment that codes for nonstructural (NSs) and nucleocapsid (NP) proteins.

2 Research Objectives

1) Assess the genetic variation of HRTV in NYS
2) Assess the potential of HRTV host-switching into *Ixodes scapularis* ticks

3 Materials and Methods

- Extract and isolate small segment (S segment) viral RNA from NY 2021 tick isolates and use next generation sequencing with the Illumina platform to analyze potential changes
- Experimentally infect *Amblyomma americanum* and *Ixodes scapularis* with HRTV via tick immersion and harvested at 7, 14, 21, and 28 days post infection (dpi) for qRT-PCR and next generation sequencing analysis

4 Results: Phylogenetic analysis of NYS HRTV isolates

- Phylogenetic analysis revealed 2018 and 2021 NYS isolates are genetically distinct
- 2021 NYS isolates are genetically closer in identity to Missouri isolates suggesting a novel introduction of the virus to NYS

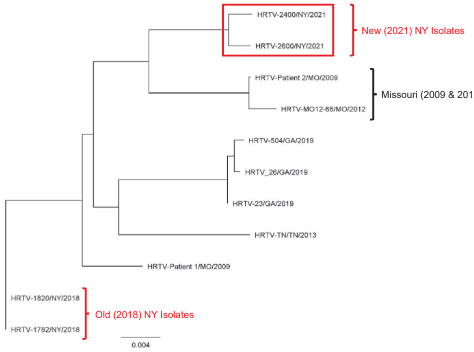


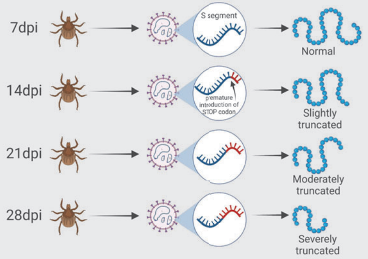
Fig 4. Phylogenetic tree of the S segment of HRTV isolates created with Geneious

CDS Region	NT Position	Consensus	2018 NY Isolates	2021 NY Isolates	Amino Acid Change
NSs	48	T	C	T	-
NSs	84	C	T	C	-
NSs	177	T	T	C	-
NSs	366	C	T	C	-
NSs	414	C	T	C	-
NSs	424	T	C	T	S142P
NSs	429	G	A	G	-
NSs	504	T	T	C	-
NSs	585	G	G	A	-
NSs	706	G	A	G	E236K
NSs	710	G	G	T	V237G
NSs	732	C	T	C	-
UTR	941	T	T	C	-
UTR	969	T	C	T	-
NP	1250	C	C	T	-
NP	1358	T	A	T	-
NP	1604	C	T	C	-
NP	1640	T	A	T	-

Fig 5. Genetic and amino acid changes in the S segment between new (2021) and old (2018) NY HRTV isolates. The dash (-) denotes a silent mutation. The yellow highlighted regions indicate an amino acid change. The reference sequence used was accession number MZ440344.1.

5 Results: Passage of HRTV in *Amblyomma americanum* and *Ixodes scapularis*

- Isolated S segment of HRTV in experimentally infected *Amblyomma americanum* 14-28dpi revealed an introduction of a nonsense mutation in the nonstructural coding region which occurred sooner in the coding region the longer the tick had been infected



- qRT-PCR analysis of experimentally infected *Ixodes scapularis* revealed no HRTV detection

6 Conclusions

- HRTV is a newly emergent tick-borne virus in NYS that has genetically and geographically expanded in recent years
- New HRTV strains form a unique cluster suggesting recent introduction from the Midwest
- Tick host may play a role in changing viral proteins in the S segment
- *Ixodes scapularis* have little to no competence for HRTV
- **Future Directions**: phenotypic characterization of newly emergent strains sequence other new isolates from NYS, sequence the other viral segments, and try experimental infection via microinjection in Ixodes scapularis

Acknowledgements

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Works Cited

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