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Ishmael D. Aziati

Derek McFarland Jr.

Avan Antia

Astha Joshi

Anahi Aviles-Gamboa

See next page for additional authors

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Authors
Ishmael D. Aziati, Derek McFarland Jr., Avan Antia, Astha Joshi, Anahi Aviles-Gamboa, Preston Lee, Houda Harastani, David Wang, Solny A. Adalsteinsson, and Adrianus C M Boon

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Original article

Prevalence of Bourbon and Heartland viruses in field collected ticks at an environmental field station in St. Louis County, Missouri, USA

Ishmael D. Aziati[a], Derek McFarland Jnr[b], Avan Antia[c], Astha Joshi[a], Anahi Aviles-Gamboa[b], Preston Lee[a], Houda Harastani[a], David Wang[c,d], Solny A. Adalsteinsson[b], Adrianus C. M. Boon[a,c,d,*]

[a] Department of Medicine, Washington University in St. Louis, USA
[b] Tyson Research Center, Washington University in St. Louis, USA
[c] Department of Molecular Microbiology, Washington University in St. Louis, USA
[d] Department of Pathology and Immunology, Washington University in St. Louis, USA

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ABSTRACT

Heartland and Bourbon viruses are pathogenic tick-borne viruses putatively transmitted by *Amblyomma americanum*, an abundant tick species in Missouri. To assess the prevalence of these viruses in ticks, we collected 2778 ticks from eight sampling sites at Tyson Research Center, an environmental field station within St. Louis County and close to the City of St. Louis, from May - July in 2019 and 2021. Ticks were pooled according to life stage and sex, grouped by year and sampling site to create 355 pools and screened by RT-qPCR for Bourbon and Heartland viruses. Overall, 14 (3.9%) and 27 (7.6%) of the pools were positive for Bourbon virus and Heartland virus respectively. In 2019, 11 and 23 pools were positive for Bourbon and Heartland viruses respectively. These positives pools were of males, females and nymphs. In 2021, there were 4 virus positive pools out of which 3 were positive for both viruses and were comprised of females and nymphs. Five out of the 8 sampling sites were positive for at least one virus. This included a site that was positive for both viruses in both years. Detection of these viruses in an area close to a relatively large metropolis presents a greater public health threat than previously thought.

1. Introduction

Emerging infectious diseases (EIDs) have become an increasing global concern in the twenty-first century; their emergence and spread pose a significant threat to global health and economies (Sabin et al., 2020). From an extensive review of the literature for EID events between 1940 and 2004, the majority (60.3%) were caused by zoonotic pathogens. Remarkably, the second (22.8%) most important category was infections caused by vector-borne diseases (Jones et al., 2008). Vector-borne infections are an important cause of morbidity and mortality with mosquitoes being responsible for most of the cases worldwide. In the United States (US) however, ticks are the leading disease vector accounting for over 90% of the annual vector-borne cases with Lyme disease being the most commonly reported (Eisen et al., 2017; Rodino et al., 2020b; Rosenberg et al., 2018). Ticks are blood-sucking arthropods that are competent vectors of a wide range of vertebrate pathogens including viruses (Rodino et al., 2020a; Tokarz and Lipkin 2020). Substantial geographic expansions of tick populations have been revealed by tick surveys throughout the US, and tick species like the lone star tick (*Amblyomma americanum*) have expanded their range in the US in recent decades (Sonenshine 2018; Molaei et al., 2019). This three-host tick is predominantly found in wooded areas especially in the presence of dense underbrush (Hair Jakie and Dariel Elza, 1970). We and others have also shown that habitats with high abundances of exotic invasive species such as bush honeysuckle (*Lonicera. maacki*) have a positive correlation to tick abundance (Adalsteinsson et al., 2016; Van Horn et al. 2018). *A. americanum* is distributed from west-central Texas to the Atlantic Coast and northward all the way to Maine, a boundary that was first reported only a decade ago, indicating an expansion of what was previously documented. Adult *A. americanum* ticks feed on medium and

* Corresponding author at: Department of Internal Medicine, School of Medicine, Washington University in St. Louis, Campus box 8051, 660 South Euclid Avenue, St. Louis, MO 63110, USA.
E-mail address: jboon@wustl.edu (A.C.M. Boon).

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large-sized mammals, and the larvae and nymphs parasitize on a wide variety of small to large mammals and ground-feeding birds (Cooley and Kohls 1944). All three motile life stages bite humans. They are not evenly distributed in nature, with high numbers occurring in relatively small geographic areas. These high population densities coupled with its aggressive and mostly non-specific feeding habitats makes *A. americanum* ticks one of the most economically important ticks in the United States (Goddard and Varela-Stokes, 2009). *A. americanum* is responsible for the continuous emergence and spread of tick-borne diseases like ehrlichiosis, tularemia and STARI (Rodino et al., 2020b). Aside from these, field isolations and laboratory studies have implicated the *A. americanum* tick as the putative vector of Heartland virus (HRTV) and Bourbon virus (BRBV), two tick-borne viruses discovered in the US in the last decade (Bosco-Lauth et al., 2016; Brault et al., 2018; Lambert et al., 2015; Newman et al., 2020; Savage et al., 2018a, 2018b). HRTV and BRBV were first isolated from febrile patients in Missouri (MO) and Kansas (KS) respectively, who reported histories of tick bites (Kosoy et al., 2015; McMullan et al., 2012). Since the discovery of these new tick-borne viruses, about 50 HRTV and 5 BRBV infections have been confirmed in human cases of febrile illness with sometimes fatal outcome across the US (Savage et al., 2017; Bricker et al., 2019; Brault et al., 2018). Similarly, these viruses have been detected in field collected *A. americanum* ticks, along with serological evidence of infection in wildlife in the Midwest and Eastern US states (Newman et al., 2020; Dupuis et al., 2021; Tuten et al., 2020; Jackson et al., 2019a; Savage et al., 2017; Savage et al., 2018a, 2018b).

BRBV is a segmented RNA virus that belongs to the family *Orthomyxoviridae* and the genus *Thogotovirus*. Members of this genus have been shown to be distributed worldwide but not until recently in the US (Savage et al., 2017; Kosoy et al., 2015; Lambert et al., 2015; Savage et al., 2018a). BRBV is the first *Thogotovirus* discovered in North America that is known to infect humans. Three other members of this genus, *Thogotovirus*, Oz virus and Dhori virus, which have not been found in the US, have been associated with human disease (Butenko et al., 1987; Frese et al., 1995; Tran et al., 2022). HRTV is also a segmented RNA virus that belongs to the family *Phenuiviridae*: genus *Bandavirus* (Savage et al., 2016; Sun et al., 2022). It is genetically closely related to Dabie bandavirus, a virus that was first identified in China in 2009 and later reported in Japan and South Korea (Brault et al., 2018; Liu et al., 2014).

The prevalence of BRBV and HRTV in field collected ticks has only been reported in the rural northwestern part of Missouri, with no peer-reviewed published reports from the other parts of the state. As part of a broader study aimed at an integrated vector-animal-human surveillance for known and novel tick-borne viruses, we tested the prevalence of BRBV and HRTV in field collected ticks from Tyson Research Center in Missouri, which is an environmental field station that is known to have abundant host-seeking *A. americanum* ticks as well as co-mingling of vectors and different animal hosts (Van Horn et al., 2018). We determined the prevalence of HRTV and BRBV by RT-qPCR in *A. americanum* sampled in 2019 and 2021 and report for the first time the surveillance and detection of these viruses in an area close to a relatively large metropolitan area.

2. Materials and methods

2.1. Study area

Tyson Research Center (TRC) is the ~800 ha environmental field station of Washington University in St. Louis, situated ~20 km southwest of the city of St. Louis, Missouri, US within the adjacent St. Louis County (38°31'N, 90°33'W). With the exception of an interstate to its southern edge, TRC is surrounded by protected lands that are popular with outdoor recreationists. TRC is located in the northeastern edge of the Ozark ecoregion and is ~85% forested, consisting of mainly deciduous oak-hickory forest on steep slopes and ridges. South-facing slopes tend to be dominated by chinquapin oak (*Quercus muehlenbergii*) and eastern red cedar (*Juniperus virginiana*); protected slopes by flowering dogwood (*Cornus florida)*, white oak (*Q. alba*), and black oak (*Q. velutina)*; and bottomlands by slippery elm (*Ulmus rubra*) and American sycamore (*Plantanus occidentalis*) (Kensinger and Allan, 2011). Forest understory contains areas of woody shrubs including spicebush (*Lindera benzoin)*, common buckthorn (*Frangula caroliniana*), and pawpaw (*Asimina triloba*). In all habitat types there are significant and patchy areas of Amur honeysuckle (*Lonicera maackii)* invasion, some of which are under ongoing management. TRC also maintains open old field habitat in its central valley and has ~24 ha of limestone/dolomite glades that are heavily invaded by red cedar. Prior to its acquisition by Washington University in St. Louis, TRC land has been used for: mining (at Mincke Quarry), logging, grazing livestock, habitation of a small village (now only ruins/building foundations remain in Mincke Valley), and construction of dozens of bunkers (which remain along the central valley) for munitions storage by the US Military. *Amblyomma americanum* is the most commonly-encountered tick species at TRC; *Dermacentor variabilis* and *Ixodes scapularis* are also present (Van Horn et al., 2018)

2.2. Tick collection and identification

Ticks were collected between May and July in 2019 and 2021 from eight different locations within TRC. These included Bunker 51, Bunker 37, Bat Road, Library Road, North Gate, Mincke Quarry, Mincke Valley and Plot 7/8 (Fig. 2b). The first five sampling locations are in the central valley and are characterized by vegetation typical of TRC protected slopes and/or bottomland forest, as described above. North Gate has extensive L. *maackii* invasion and is distinct from the other valley locations for its close proximity to the Meramec River. Mincke Quarry has been modified by mining activities and has mostly shallow soils, exposed slopes, *J. virginiana* and L. *maackii* invasion. Mincke Valley contains remnants of old building foundations but is otherwise closed-canopy oak-hickory forest with understory dominated by *A. triloba* and L. *maackii*. Plot 7/8 is on a steep west-facing slope dominated by oak-hickory forest. Ticks were captured using a combination of standard drag sampling, flagging, and dry ice trapping methods, as previously described (Savage et al., 2013).

Field collected ticks were transported alive to the TRC laboratory and frozen at −80 °C. There, ticks were identified and sorted according to species, sex, and life stage on a cold tray under a dissecting microscope using taxonomic keys (Keirans and Durden, 1998; Keirans and Litwak, 1989). Ticks were then grouped into pools (up to $n = 5$ for adult ticks and $n = 25$ for nymphs) by location, sampling date, species, sex and life stage. The pools were stored at −80 °C and transported on dry ice to Washington University School of Medicine for further processing.

2.3. RNA extraction and virus detection

1.0 mL of cold Trizol reagent was added (Invitrogen, cat # 15596018) to a 2 mL homogenization tube containing three stainless steel beads and the pooled ticks. For every five pools, we added a negative control tube (without ticks) as a control for cross contamination. The six tubes were then placed in the TissueLyser II (Qiagen Cat. No. / ID: 85300) and run at 30 Hz (1800 oscillations/minute) at 30 s interval until ticks were well homogenized. The homogenates were then subjected to RNA extraction using the manufacturer's protocol. The extracted RNA was stored at −80 °C until ready to be used. The samples were screened separately in a one-step RT-qPCR for the presence of BRBV and HRTV viral RNA. We used published primers and probes targeting BRBV nucleoprotein (BRBV NP) (Lambert et al., 2015) and HRTV small non-structural protein (HRTV NSs) (Savage et al., 2013) as screening primers (Table 1). Confirmatory primer probe sets targeting different genes (BRBV PB1 and HRTV NP) (Table 1) of the viruses were used to re-test the positive pools. A *de novo* A. americanum tick 16S mitochondrial rRNA (Tick 16S) primer/probe was used as internal

Table 1
Real-time PCR primer/probes for the detection of BRBV, HRTV and tick 16S rRNA.

Gene	Fwd Primer Sequence	Probe Sequence	Rev. Primer Sequence	Refs.
BRBV NP	GCAAGAAGAGGCCAGATTTC	CCTCACACCACGGAAGCTGGG	TCGAATTCGGCATTCAGAGC	Lambert et al. (2015)
HRTV NSs	TGCAGGCTGCTCATTTATTC	CCTGACCTGTCTCGACTGCCCA	CCTGTGGAAGAAACCTCTCC	Savage et al. (2013)
Tick 16S	ACTCTAGGGATAACAGCGTAATAA	TGCGACCTCGATGTTGGATTAGGA	CTGAACTCAGATCAAGTAGGACA	This study
Confirmatory Primer/Probes				
BRBV PB1	AACCGAAGGACCATTGCTAC	ACCCTTGCTGCATCTTCCACCA	ACAGGGACTCCAGAACTTGG	Lambert et al. (2015)
HRTV NP	CCTTTGGTCCACATTGATTG	TGGATGCCTATTCCCTTTGGCAA	CACTGATTCCACAGGCAGAT	Savage et al. (2013)

control which served as an indicator for successful tick homogenization and RNA extraction. An experiment (sets of 5 samples plus 1 negative control) was considered valid if the negative control (samples extracted without ticks) had a Ct value of undetermined, and pools were scored as positive if it had a Ct value equal to or less than 35 (Ct $\leq$ 35) for both 16S and virus-specific primers.

2.4. Quantification by real-time RT-qPCR

In vitro transcribed RNAs encompassing the BRBV NP, HRTV NSs, and Tick 16S target sequences were used to define the efficiency and limit of detection of the assay and quantify the number of genome copies per sample. Standards were diluted to obtain 2.5×10^7 RNA copies/μL and this was 10-fold serially diluted to 0.025 copies/μL. 4 μL of the diluted standards was used to generate standard curves in a quantitative RT-PCR (RT-qPCR) employing the following conditions; 48 °C for 15 min, 95 °C for 10 min, (95 °C for 15 s, 60 °C for 1 min) X 50 cycles on the QuantStudio 6 flex Real-time PCR system (Applied Biosystems), using the TaqMan RNA-to-CT 1-step kit (Applied Biosystems).

2.5. Maximum likelihood estimate (MLE) of infection rate (IR)

Maximum likelihood estimate (MLE) of the infection prevalence per 1000 ticks at 95% confidence intervals (CIs) for the infection rate were computed using the Excel Add-In (Biggerstaff, 2006).

3. Results

3.1. Evaluation of RT-qPCR primers and probes for BRBV and HRTV

To define the sensitivity of these primers, we used serial dilutions of the *in vitro* transcribed target RNA sequences standard as template and reliably detected 10 RNA copies in the reaction at an average Ct value of 36 for BRBV NP and 16S primer/probe sets, and 35 for HRTV S (Fig. 1a). In order to mimic physiological conditions and to determine the presence of RT-qPCR inhibitors in the tick homogenates, we spiked RNA extracted from a BRBV/HRTV-negative tick pool with our RNA standard and generated a standard curve as above and still detected 10 RNA copies at an average Ct value of 36 for BRBV NP and 16S and 35 for HRTV S (Fig. 1b), suggesting that RNA from homogenized ticks does not inhibit the RT-qPCR reaction. Based on this data, we set the cut off Ct value for a positive sample at $\leq$ 35.

3.2. Tick collection

Ticks were collected in 2019 ($n = 1556$) and in 2021 ($n = 1222$) from eight different locations at TRC. *A. americanum* accounted for nearly 99.4% of the collected ticks at TRC in both 2019 and 2021 collections. The remaining 0.6% were *Dermacentor variabilis*. In the 2019 collection, 29% of *A. americanum* ticks were adult males, 33% were adult females, and 38% were nymphs. In the 2021 collection, 11% were adult males, 16% were adult females, 13% larva, and 60% were nymphs (Fig. 2a).

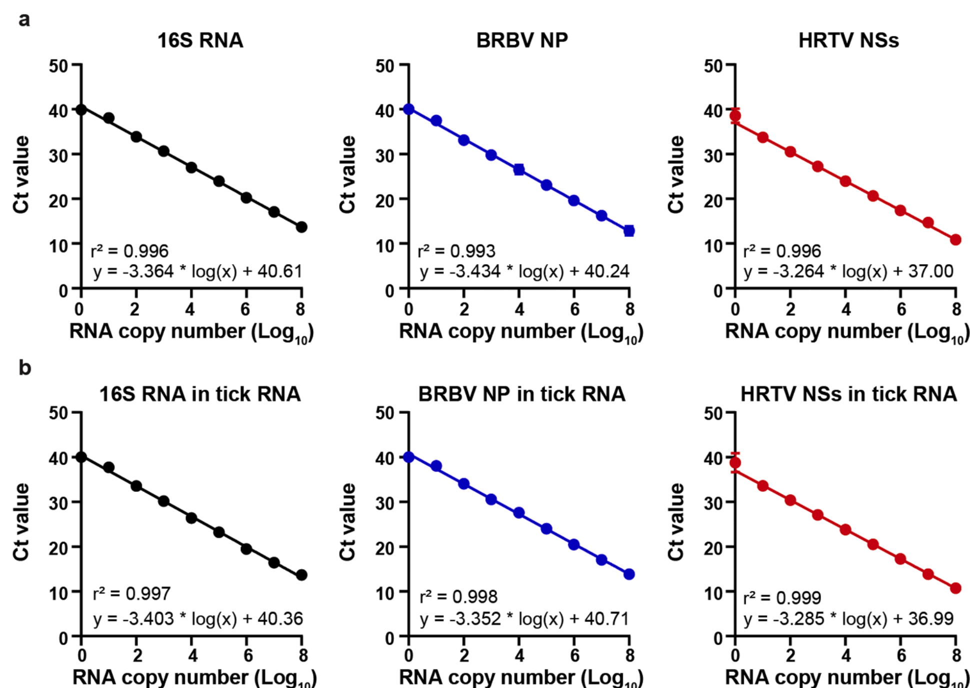

Fig. 1. Sensitivity of Primer/Probes in detecting *in vitro* transcribed RNA Standard. The primer/probe sets 16S (*Left*), BRBV NP (*Middle*) and HRTV NSs (*Right*) were used to generate a standard curve in RT-qPCR using a 10 fold serially diluted *in vitro* transcribed RNA as template (a) or tick RNA spiked with *in vitro* transcribed RNA (b).

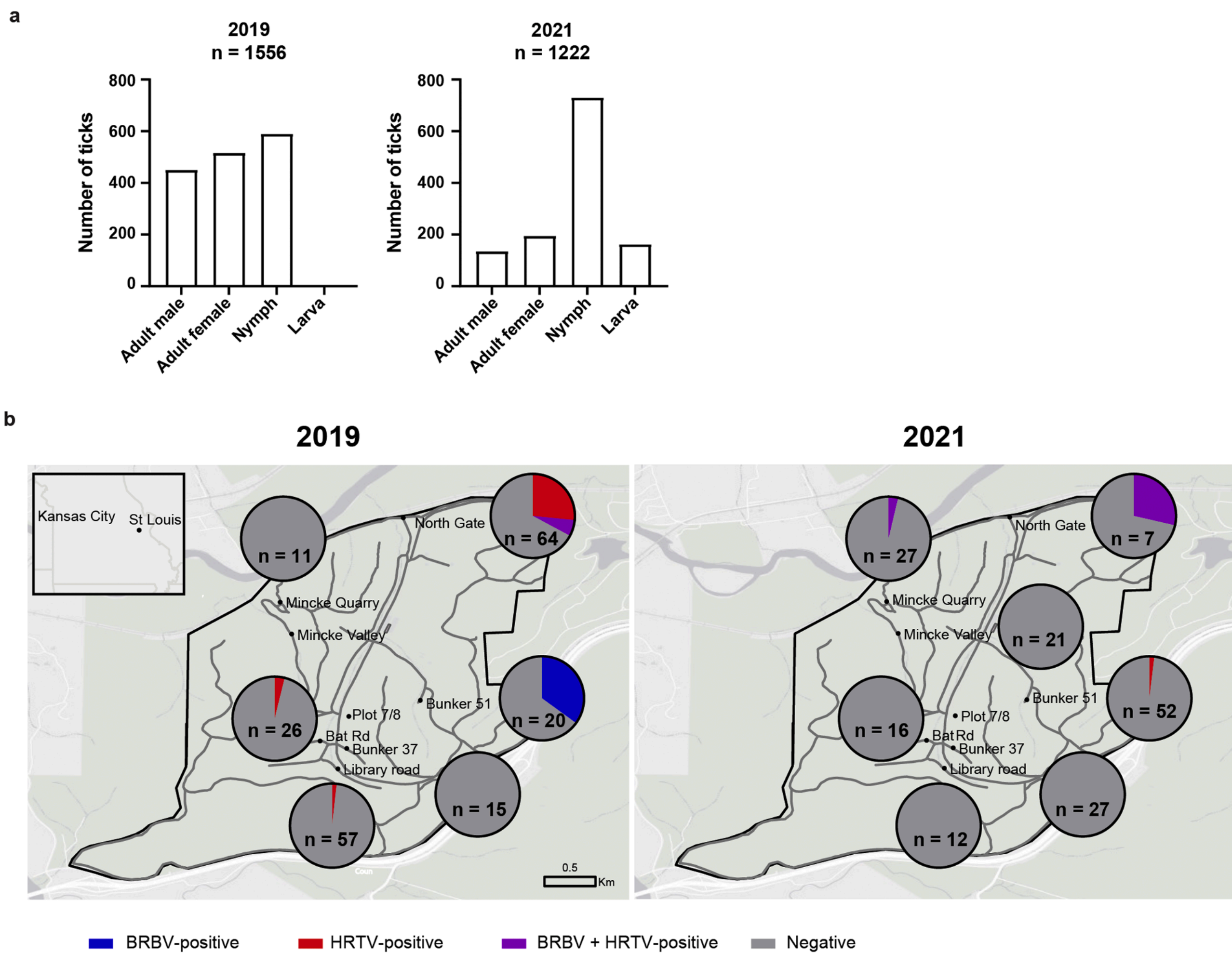

Fig. 2. Number of ticks collected in study, grouped according life stage and sex. Number of ticks collected in study, grouped according life stage and sex in 2019) Fig. 2a (left panel) and 2021 (right panel) Fig. 2b. Positivity of BRBV and HRTV by location during 2019 and 2021 collections. Map of TRC showing 8 approximate tick sampling locations during 2019 and 2021 sampling seasons. In 2019, the locations sampled were Bunker 51, Bat Road, Bunker 37, North Gate, Mincke Quarry and Library Road. In 2021, we sampled all 2019 locations except Bunker 37, and two new locations – Mincke Valley and Plot 7/8. The pie charts represent the number of pools positive for BRBV (blue), HRTV (red), BRBV & HRTV (purple), or negative (gray), and the geographical location of these virus positive pools. The tick pool number is shown inside the pie charts. The black outline shows the TRC property boundary and gray lines depict paved and gravel roads within TRC property. The insert map is that of US State of Missouri with the location of TRC (black dot) relative to Kansas City, and St. Louis city. Maps were created using ArcGIS Pro v2.8 ('Citation: ESRI 2021. ArcGIS Pro version 2.8. Redlands, CA: Environmental Systems Research Institute') with included area basemap and spatial data collected at TRC.

3.3. Detection of BRBV and HRTV in ticks

In 2019, 11 and 23 pools out of the 193 tested were BRBV and HRTV positive respectively. Seven of the BRBV positive pools were from Bunker 51 and ticks were collected on the 23rd of May, and the remaining 4 BRBV positive pools were made up of ticks collected on the 10th of June at North Gate. Of the 11 BRBV positive pools, 8 were pools of adult males, 2 of adult females and 1 of nymphs. Of the 23 HRTV positive pools, 21 were from North Gate and one each from Library Road and Bat Road collected on the 10th, 3rd and 4th of June respectively. The HRTV positive pools included 20 pools of adult males, 1 of adult females and 2 of nymphs (Fig. 2b, Table 2). The infection rates (IRs) for

Table 2
Information on the virus positive pools in the 2019 collection.

Location	Male			Female			Nymph			Total	
	HRTV +	BRBV +	# pools	HRTV +	BRBV +	# pools	HRTV +	BRBV +	# pools	# + pools	# pools
Bunker 51	0	6	11	0	1	9	0	0	0	7	20
Bat Road	1	0	13	0	0	13	0	0	0	1	26
Bunker 37	0	0	7	0	0	5	0	0	3	0	15
North Gate	19	2	27	1	1	25	1	1	12	*21	64
Mincke Quarry	0	0	6	0	0	1	0	0	4	0	11
Library Road	0	0	27	0	0	26	1	0	4	1	57
Total pools	20	8	91	1	2	79	2	1	23	30	193
Indv. Ticks	450	516	590	1556							

*4 pools were positive for both Bourbon and Heartland viruses.

A. americanum among the virus positive locations for BRBV were 7.0 (CI = 2.3 - 16.7) for North Gate and 80.6 (CI = 36.5 – 155.4) for Bunker 51. The HRTV IRs were, 39.5 (CI = 26.6 – 57.2) for North Gate, 8.1 (CI = 0.47 – 38.6) for Bat Road, and 2.8 (CI = 0.16 – 13.7) for Library Road. We also calculated IRs of BRBV and HRTV in the ticks by life stage and sex. The BRBV and HRTV IRs for all adults (male and female) *A. americanum* were 10.5 (5.4 – 18.5) and 22.6 (14.6 – 33.6) respectively. The BRBV IRs for *A. americanum* adult male, adult female and nymph in the entire 2019 collection were 18.3 (8.6 – 34.5), 3.8 (0.70 – 12.5) and 1.7 (0.10 – 8.3) respectively. HRTV IRs were 48.8 (31.0 – 73.4), 1.9 (0.1 – 9.2) and 3.4 (0.6 – 11.4) for male, female and nymph respectively.

In 2021, there were four virus positive pools out of a total of 162 tested. One pool was HRTV positive and the remaining three were positive for both viruses. The virus positive pools were from three locations: Bunker 51 (1/4); North Gate (2/4); and Mincke Quarry (1/4) (Fig. 2b and Table 3). The ticks were collected on 4th of June and 9th of July. The IRs of BRBV in ticks by life stage and sex were 10.3 (1.85–33.2) for adult female, 1.3 (0.008–6.5) for nymph, and zero for adult male and larvae. The IRs for HRTV in all adults (male and female) *A. americanum* were 6.0 (1.0–19.6) and 2.7 (0.5–8.9) for nymphs.

The BRBV IRs for *A. americanum* adult male, adult female and nymph in the entire study (2019 & 2021) were 14.0 (6.6–26.4), 5.6 (1.8–13.4) and 1.5 (0.2–4.9), respectively and for HRTV for the same period were 36.7 (23.2–55.3), 4.2 (1.1–11.3) and 3.1 (1.0–7.4).

The Ct value for the positive pools ranged from 24.58–34.65 corresponding to 6.6×10^4 – 67 RNA copies for BRBV and 23.05–35.04 corresponding to 2.0×10^4 – 5.6 RNA copies for HRTV.

To validate and confirm our results, twelve BRBV positive pools were re-tested with a primer probe set specific for a different gene of BRBV (PB1). All 12 samples also tested positive for BRBV PB1 with similar Ct values compared to the BRBV NP RT-qPCR. We also re-tested 20 HRTV-positive pools with a primer probe set that targets the NP gene of HRTV. Over 75% (16/20) of the positive pools were also HRTV positive with the second primer probe set.

4. Discussion

In this study, we report a high prevalence of BRBV and HRTV viruses in field collected ticks at an environmental field station in the eastern-central part of MO at the edge of the St. Louis metropolitan area. A total of 2778 ticks were collected between May - July of 2019 and 2021, and we detected BRBV and HRTV viral RNA in tick homogenates by RT-qPCR. Thirty-four RT-qPCR virus-positive pools were obtained from five of the eight sampled locations (Fig. 2b) and were composed of adult (male and female) and nymphal *A. americanum* ticks.

Detection of HRTV and BRBV in adult and nymphal *A. americanum* ticks in our study is consistent with prior studies (Savage et al., 2017, 2013, 2016, 2018a, 2018b) and support the notion that *A. americanum* is a vector for HRTV and BRBV to humans. The fact that viruses were detected in both nymphal and adult stages exhibiting host-seeking behavior suggest that both life stages could potentially transmit the virus to humans. Host-seeking nymph and adult A. *americanum* ticks, likely acquire the virus during co-feeding with other infected ticks or by feeding on viremic vertebrate hosts. Transmission could be transstadial, from infected nymphs to adults or from larvae to nymphs, although screening 162 larvae (the life stage with the smallest sample size in our study), did not result in the detection of virus (Godsey et al., 2021, 2016).

BRBV and HRTV were detected at North Gate in adult and nymphal *A. americanum* for both years in our study (Fig. 2b, Tables 2 and 3) suggesting that these viruses are maintained in the tick and/or host populations in this area of TRC and that an overlap in BRBV and HRTV transmission cycles exist at this location.

Increase in HRTV incidence compared to BRBV in our study is similar to previous studies performed in Northwestern Missouri (Savage et al., 2017, 2013, 2016) and Kansas (Savage et al., 2018a, 2018b). Differences in transstadial, co-feeding, and transovarial transmission rates between HRTV and BRBV may contribute to this. Studies by Godsey et al. (2021, 2016) and colleagues showed that transstadial and co-feeding are the main routes of transmissions for BRBV with infection rates between 50 and 100%. Transovarial transmission of BRBV was observed in 5–12% of the ticks. In contrast, transovarial and transstadial transmissions of HRTV were 22–40% while co-feeding transmission was 0.2%. These results indicate that transovarial transmission of HRTV is more efficient and less dependent on co-feeding on a virus-infected host compared to BRBV, which could favor a higher prevalence of HRTV in ticks. Alternatively, differences in seroprevalence and host species between BRBV and HRTV (Riemersma and Komar, 2015; Jackson et al., 2019b) may contribute to a higher incidence of HRTV in ticks.

We observed a higher prevalence of BRBV and HRTV in ticks in our study compared to previous studies performed in and around Missouri (Savage et al., 2017, 2013, 2016, 2018a, 2018b; Tuten et al., 2020; Dupuis et al., 2021; Newman et al., 2020). We believe that this high incidence is valid and not caused by methodological biases. First, we included one negative control sample for every five tick pools and all of the negative controls had Ct values that were undetermined ($C_t > 50$). Second, the presence of HRTV or BRBV RNA in virus-positive pools were confirmed by another investigator in the lab and by a second primer probe set against a different viral gene. Third, HRTV and BRBV positive samples were processed and tested on different days and years of the study, further reducing the possibility that these samples were contaminated. That said, we were also surprised by the high infection rates in our study. One possible explanation is that we sampled fewer ticks in a relative small area compared to other studies, and that we were fortunate to sample a site (North Gate) with very high incidence of

Table 3
Information on the virus positive pools in the 2021 collection.

Location	Male			Female			Nymph			Larva			Total	
	HRTV +	BRBV +	# pools	HRTV +	BRBV +	# pools	HRTV +	BRBV +	# pools	HRTV +	BRBV +	# pools	# + pools	# pools
Bunker 51	0	0	12	0	0	21	1	0	19	0	0	0	1	52
Bat Road	0	0	5	0	0	7	0	0	4	0	0	0	0	16
North Gate	0	0	2	1	1	2	1	1	3	0	0	0	*2	7
Mincke Quarry	0	0	6	1	1	4	0	0	8	0	0	9	*1	27
Library Road	0	0	6	0	0	5	0	0	1	0	0	0	0	12
Plot 7/8	0	0	4	0	0	10	0	0	13	0	0	0	0	27
Mincke Valley	0	0	5	0	0	6	0	0	10	0	0	0	0	21
Total Pools	0	0	40	2	2	55	2	1	58	0	0	9	4	162
Indv. Ticks	135	196	729	162	1222									

*3 pools were positive for both Bourbon and Heartland viruses.

HRTV and BRBV, increasing the overall infection rate of the study. Varied infection rates among collection sites and between collection years has been reported by others (Savage et al., 2017, 2013, 2016, 2018a, 2018b).

A limitation of our study is the heterogeneity in sampling depth per location. In addition, because the whole tick was homogenized in Trizol for RNA purification, we could not culture live virus from these homogenates.

In conclusion, the infection rates per 1000 ticks varied among collection sites, life stages, sex, and between collection years. Additional tick collection efforts combined with animal and human sero-surveillance is important in gaining a deeper understanding of the environmental determinants of vector positivity and risk of exposure of these emerging viruses to humans in the US.

CRediT authorship contribution statement

Ishmael D. Aziati: Conceptualization, Methodology, Validation, Formal analysis, Investigation, Data curation, Writing – original draft. **Derek McFarland Jnr:** Investigation, Methodology. **Avan Antia:** Investigation, Writing – review & editing. **Astha Joshi:** Investigation. **Anahi Aviles-Gamboa:** Investigation. **Preston Lee:** Investigation. **Houda Harastani:** Methodology. **David Wang:** Supervision, Conceptualization, Resources, Funding acquisition, Project administration, Writing – review & editing. **Solny A. Adalsteinsson:** Supervision, Conceptualization, Formal analysis, Funding acquisition, Project administration, Writing – review & editing. **Adrianus C.M. Boon:** Conceptualization, Data curation, Supervision, Formal analysis, Funding acquisition, Project administration, Writing – review & editing.

Data availability

Data will be made available on request.

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