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Prevalence of *Ehrlichia chaffeensis* and *Ehrlichia ewingii* in *Amblyomma americanum* and *Dermacentor variabilis* collected from southeastern Virginia, 2010–2011

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Abstract

Amblyomma americanum is the most commonly-encountered tick species in southeastern Virginia, representing approximately 95% of the human-biting tick population in this area. Here we investigated the prevalence of *Ehrlichia chaffeensis* and *Ehrlichia ewingii* in questing *Amblyomma americanum* and *Dermacentor variabilis* ticks collected from multiple sites in southeastern Virginia from 2010–2011. Although both *Ehrlichia* species were detected in *Amblyomma americanum*, no evidence of either pathogen was found in *Dermacentor variabilis*. Prevalence of *E. chaffeensis* varied by location, ranging from 0 – 5.08% among *Amblyomma americanum* populations. *Ehrlichia ewingii* prevalence was slightly higher, ranging from 0 – 8.20% among *A. americanum* populations. We conclude that both pathogens are established in southeastern Virginia *A. americanum* populations, and that although there are no apparent temporal trends in *Ehrlichia* prevalence, there is variation among locations, suggesting the potential for disease hotspots.

Keywords

Amblyomma americanum; *Dermacentor variabilis*; *Ehrlichia chaffeensis*; *Ehrlichia ewingii*

Introduction

The lone star tick, *Amblyomma americanum* (L.) (Acari: Ixodidae), is found throughout the southeastern United States with populations extending west to central Texas and north to Iowa (Childs and Paddock, 2003). The eastern range of *A. americanum* extends through the mid-Atlantic region, with populations intermittently reported in New England states including Maine (Kierans and Lacombe, 1998), Connecticut and Rhode Island (Ijdo et al., 2000). *Amblyomma americanum* is the most commonly reported tick species collected from humans in the southeastern and mid-Atlantic U.S., representing over 60% of ticks collected from humans from New Jersey, Maryland, Virginia, Kentucky and South Carolina from 2004–2010 (Stromdahl and Hickling, 2012). In southeastern Virginia, *A. americanum* is the most commonly encountered human-biting tick, constituting over 95% of questing ticks collected from 2010–2012 (Nadolny et al., 2014). Because of the abundance of this tick in

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the southeastern U.S. and its propensity to feed on humans, pathogens transmitted by *A. americanum* pose an important threat to human health.

Ehrlichia chaffeensis and *Ehrlichia ewingii* are the causative agents of human ehrlichiosis and are transmitted to humans and animals by infected *A. americanum* (Anziania et al., 1990 and Ewing et al., 1995). These *Ehrlichia* spp. have also been found in the American dog tick, *Dermacentor variabilis* (Say) (Murphy et al., 1998 and Steiert and Gilfoy, 2002), although it is unclear whether *D. variabilis* is capable of transmitting these pathogens. Here we describe the prevalence of *Ehrlichia chaffeensis* and *Ehrlichia ewingii* in ticks collected from southeastern Virginia.

Materials and Methods

Questing adult and nymphal *A. americanum* and adult *D. variabilis* were collected on flags from April through September of 2010 and 2011 from multiple locations representing 11 independent cities and counties in southeastern Virginia (Fig. 1). Nine sites were sampled on a weekly basis in 2010 and 12 sites were sampled on a weekly basis in 2011 (Nadolny et al., 2014). Within each site, the area of each transect was recorded so that density of host-seeking ticks encountered during each sampling event could be determined. Ticks were identified to species morphologically (Keirans and Litwak, 1989; Keirans and Durden, 1998) and individuals were pooled prior to DNA extraction. Adult *A. americanum* and *D. variabilis* collected at the same location in the same week were morphologically identified and then pooled into groups of up to 10. *Amblyomma americanum* nymphs were pooled into groups of up to 25. Prior to extraction all adult ticks were cut in half, one half was used for DNA extraction and the other stored at −80°C for future use. All ticks were homogenized by bead-beating with 1 mm glass beads. DNA was extracted using the DNeasy Blood and Tissue Kit (Qiagen, Inc. Valencia, CA) following the manufacturer's protocol and stored at −20°C.

Samples were tested separately for *E. chaffeensis* and *E. ewingii* DNA using real-time quantitative PCR (qPCR) assays specific to each species. Both *E. chaffeensis* and *E. ewingii* were tested for using TaqMan qPCR assays targeting the 16S rRNA gene (Loftis et al., 2003 and Killmaster et al., 2014). A subset of qPCR-positive samples were confirmed by sequencing either the groEL gene of *E. chaffeensis* (Tabara et al., 2007) or the p28 gene of *E. ewingii* (Gusa et al., 2001) using a nested PCR assay. A total of 38 *E. chaffeensis* positive samples and 6 *E. ewingii* positive samples were sequence-confirmed. Sequences were analyzed by performing a BLAST search on GenBank.

Because tick samples were pooled prior to extraction, a maximum likelihood estimation (MLE) was used to approximate the true prevalence of *E. chaffeensis* and *E. ewingii* in the tick population. The software used to perform MLE (Biggerstaff, 2008) was acquired from the Centers for Disease Control and Prevention website (CDC).

Results

A total of 605 *D. variabilis* adults and 8700 *A. americanum* adults and nymphs were collected during 2010 and 2011. The highest numbers of both species were collected in May

and June in both years (Fig. 2). Although both *E. chaffeensis* and *E. ewingii* were detected in *A. americanum*, no evidence of either pathogen was found in the *D. variabilis* tested. Sequence confirmation of 44 positive samples showed either ≥99% match to *E. chaffeensis* or 100% match to *E. ewingii* in a BLAST search. A total of 967 and 981 *A. americanum* pools were tested for *E. chaffeensis* and *E. ewingii*, respectively. Because testing for each pathogen was performed at different times, not every sample was available for testing in both assays. Overall prevalence of *E. chaffeensis* in *A. americanum* adults and nymphs was 0.9% in 2010 and 0.6% in 2011; *E. ewingii* prevalence was 1.5% and 1.3% in 2010 and 2011, respectively (Table 1). A higher prevalence of both *Ehrlichia* spp. was found in adults than in nymphs, with adults having an approximate ten-fold greater prevalence of both pathogens (Table 1). In adults, prevalence of *E. chaffeensis* varied by location (Table 2), ranging from 0 – 4.3% (mean = 1.6 ± 1.4) in 2010 and 0 – 5.1% (mean = 1.1 ± 1.6) in 2011. Prevalence of *E. ewingii* in adults also varied by location, ranging from 0 – 8.2% (mean = 3.1 ± 2.6) in 2010 and 0 – 7.7% (mean = 2.8 ± 2.8) in 2011. The higher prevalence of *E. ewingii* relative to that of *E. chaffeensis* in adult *A. americanum* was mainly driven by one site in Virginia Beach, which had the highest prevalence of all sites (8.2% in 2010 and 7.7% in 2011). Although greater numbers of both *A. americanum* and *D. variabilis* were collected during May and June, there were no apparent spatial or temporal trends in prevalence of either *Ehrlichia* spp. (Table 2).

To validate the accuracy of the maximum likelihood estimation, leftover individual adult *A. americanum* halves from pools which tested positive for *E. chaffeensis* were extracted and tested by qPCR for *E. chaffeensis*. A MLE was then performed to determine *E. chaffeensis* prevalence within these individually extracted samples. Pooled samples had an overall *E. chaffeensis* prevalence of 1.95% in 2010, whereas when MLE analysis of individually extracted samples indicated a prevalence of 2.01%. Since these prevalence values are similar, this experiment validates the accuracy of the MLE calculation, which has been used extensively to estimate the prevalence of vector-borne disease agents in studies with pooled samples.

Discussion

We describe the collection of both *A. americanum* and *D. variabilis* in southeastern Virginia, as well as the presence of both *E. chaffeensis* and *E. ewingii* in questing *A. americanum* nymphs and adults. Although *D. variabilis* has occasionally been shown to harbor these pathogens, we found no evidence of either pathogen in any *D. variabilis* collected in this study. The prevalence of *E. chaffeensis* (1.4 – 2.0%) and *E. ewingii* (3.4 – 3.5%) in adult *A. americanum* is comparable to the prevalence of *E. chaffeensis* (2.2%) and *E. ewingii* (2.2%) determined in another study assessing the rate of *Ehrlichia* spp. infection in *A. americanum* adults collected in 2012 (Gaines et al., 2014). The study, which assessed the prevalence of *Ehrlichia* spp. in *A. americanum* adults collected throughout the state of Virginia, found that *E. chaffeensis* prevalence ranged from 0 – 24.5% and *E. ewingii* prevalence ranged from 0 – 14.3% (Gaines et al., 2014). The lower infection prevalence in *A. americanum* nymphs is consistent with other studies assessing the prevalence of *Ehrlichia* spp. in questing ticks. *Amblyomma americanum* nymphs collected in Maryland were

determined to have an *E. chaffeensis* minimum infection rate (MIR) of just 0.8%, while adults showed a MIR prevalence of 3.5% (Stromdahl et al., 2000). Given that the white-tailed deer (*Odocoileus virginianus*) is a known reservoir of *Ehrlichia* spp. (Ewing et al., 1995, Lockhart et al., 1997) it is not surprising that questing *A. americanum* adults, which have taken two bloodmeals in their lifetime, would have a greater prevalence than questing nymphs, which would have taken just one bloodmeal.

Other studies have noted the great abundance of *A. americanum* present in the southeastern and south-central United States in relation to other sympatric tick species (Stromdahl and Hickling, 2012 and Nadolny et al., 2014). All three *A. americanum* life stages (larva, nymph and adult) are known to aggressively parasitize humans and multiple concurrent tick bites of this species are often reported. Stromdahl and Hickling (2012) observed that approximately 15% of persons submitting *A. americanum* to the DOD for testing submitted multiple specimens. Because of the high proportion of *A. americanum* in this area and the propensity of this species to seek out human hosts in both the nymphal and adult stages, pathogens present even in low numbers within these populations warrant attention as concerns to public health. Furthermore, this study found no uniformity in geographic distribution of either *Ehrlichia* species in *A. americanum*, indicating a potential for disease "hotspots" in areas where these pathogens are more abundant.

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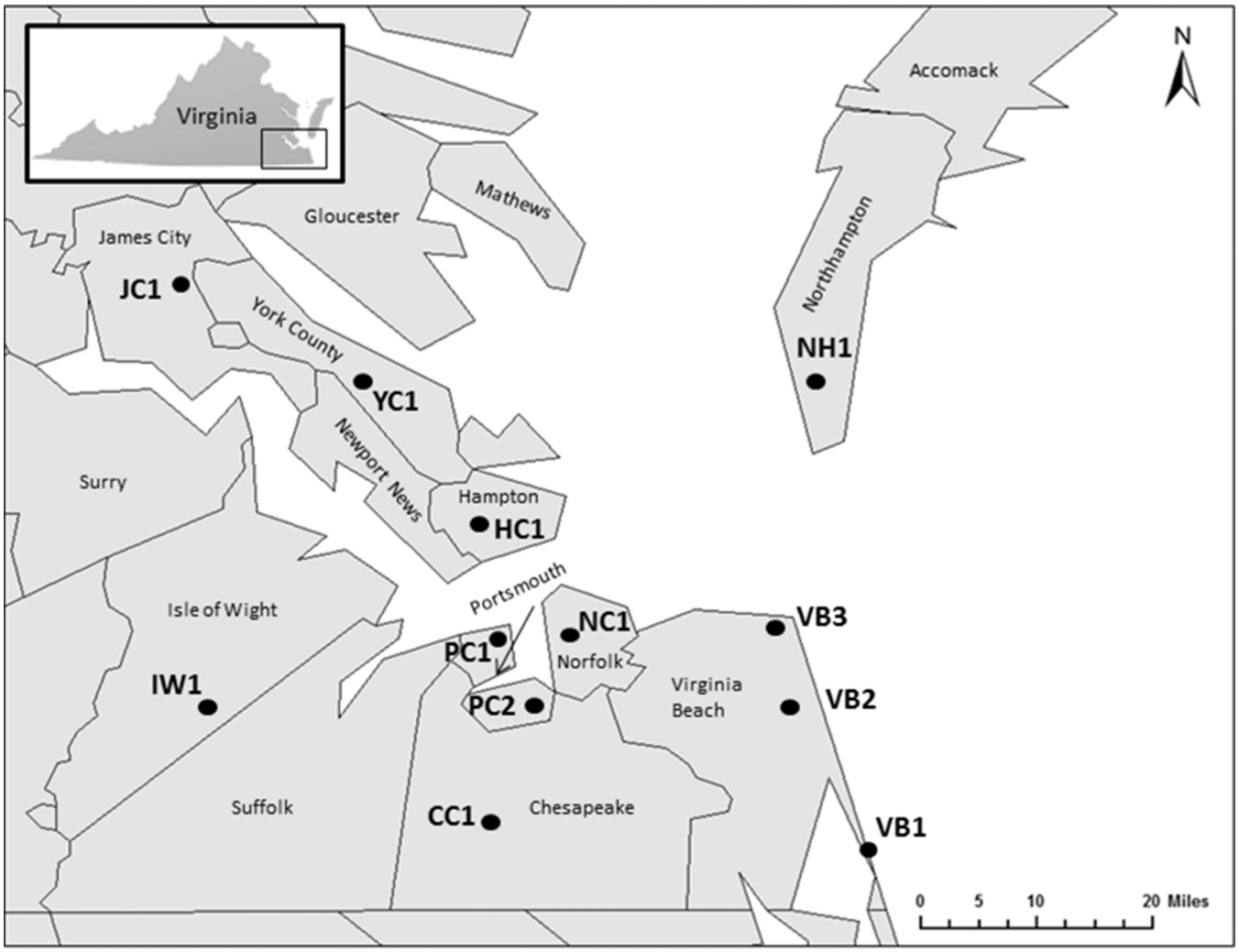

Figure 1.

Map of southeastern Virginia showing the location of the sites where ticks were collected in 2010 and 2011.

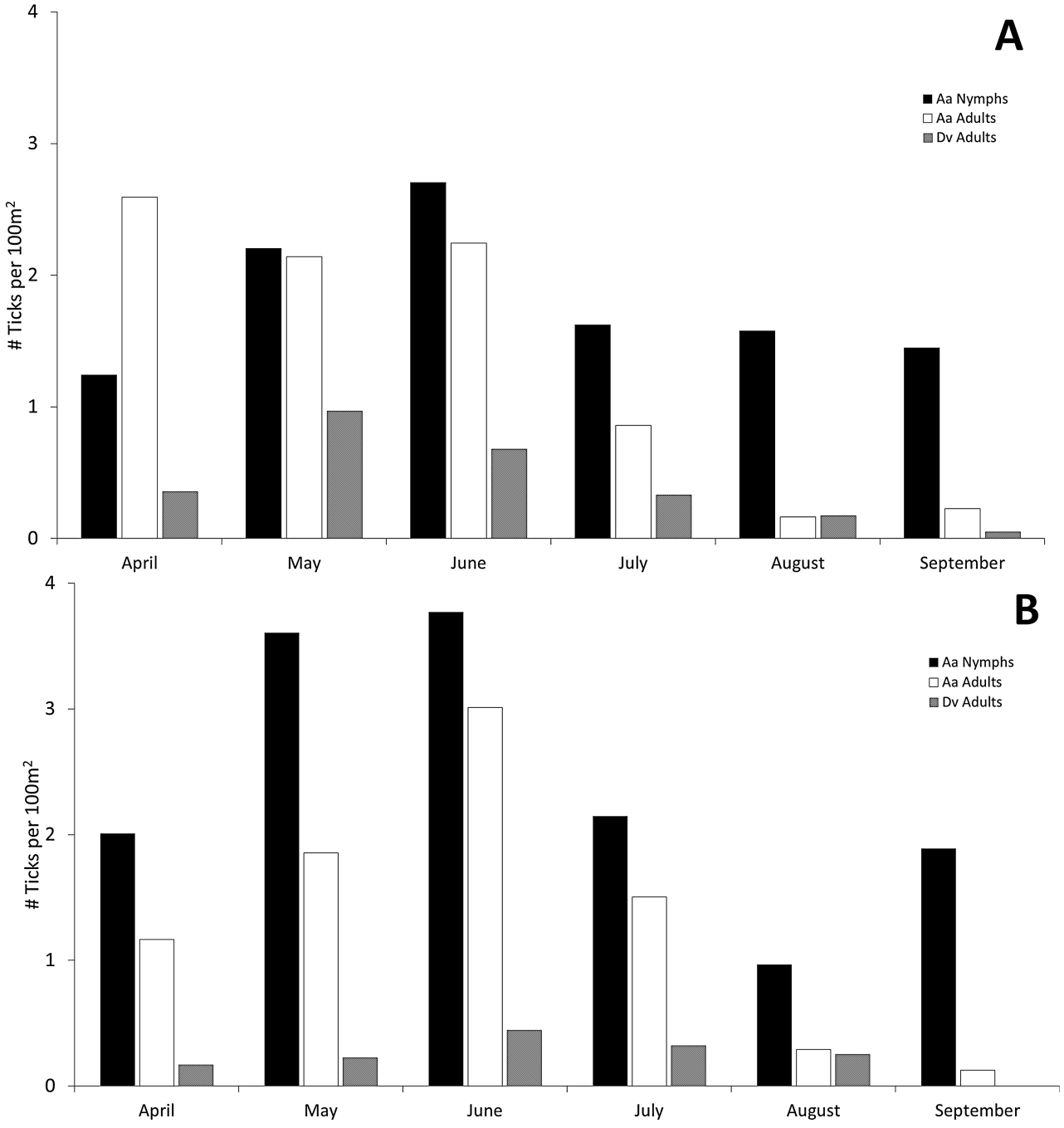

Figure 2.

Phenology of *A. americanum* nymphs and adults and *D. variabilis* adults collected during 2010 (A) and 2011 (B) from southeastern Virginia.

Table 1

Pooled and maximum likelihood estimated (MLE) prevalence of *Ehrlichia* spp. in questing adult and nymphal *A. americanum* and adult *D. variabilis* collected on flags from multiple sites within southeastern Virginia. To assess true pathogen prevalence from pooled DNA samples, a MLE calculation was used (Biggerstaff, 2008).

Tick	Year	Organism	Life Stage	Number Pools Positive	Number Pools Tested	# of Ticks	Pools Positive (%)	MLE (%)
A. americanum	2010	*E. chaffeensis*	All	28	417	3134	6.7	0.9
			Adult	25	221	1363	11.3	2.0
			Nymph	3	196	1771	1.5	0.2
		E. ewingii	All	45	426	3095	10.5	1.5
			Adult	42	231	1343	18.2	3.4
			Nymph	3	195	1752	1.5	0.2
	2011	*E. chaffeensis*	All	26	550	4814	4.7	0.6
			Adult	20	254	1483	7.8	1.4
			Nymph	6	296	3331	2.1	0.2
		E. ewingii	All	59	555	4813	10.6	1.3
			Adult	48	262	1532	18.3	3.5
			Nymph	11	293	3281	3.2	0.3
D. variabilis	2010	*E. chaffeensis*	Adult	0	69	259	0	-
		E. ewingii	Adult	0	69	259	0	-
	2011	*E. chaffeensis*	Adult	0	76	228	0	-
		E. ewingii	Adult	0	76	228	0	-

Table 2

Maximum likelihood estimated (MLE) prevalence of *Ehrlichia chaffeensis* (left) and *Ehrlichia ewingii* (right) in adult and nymphal *A. americanum* collected from various locations (Fig. 1) within southeastern Virginia in 2010 and 2011. The total number of individuals represented within pooled DNA samples is indicated.

Year	Site	*Ehrlichia chaffeensis*				*Ehrlichia ewingii*			
		MLE Prevalence (%)		# Ticks Represented		MLE Prevalence (%)		# Ticks Represented	
		Adults	Nymphs	Adults	Nymphs	Adults	Nymphs	Adults	Nymphs
2010	All	1.95	0.17	1363	1771	3.44	0.17	1343	1752
	CC1	4.33	0.85	50	121	3.50	0	59	99
	IW1	2.43	0	40	52	2.40	0	41	53
	JC1	1.09	0	92	155	5.80	0	82	153
	NH1	0	0	15	3	0	0	15	3
	PC1	2.23	0.27	630	746	2.10	0.13	624	733
	PC2	0	0	21	0	0	0	21	0
	VB1	1.04	0	93	183	3.50	0	91	185
	VB2	1.30	0	239	302	8.2	0.65	233	317
	YC1	2.30	0	183	208	2.40	0	177	208
2011	All	1.41	0.18	1483	3331	3.47	0.34	1532	3281
	CC1	0	0	203	495	2.09	0.41	203	495
	HC1	0	0	15	33	2.22	0	95	41
	IW1	5.08	0.65	41	152	5.58	0	38	48
	JC1	0	0	22	31	0	0	22	31
	NC1	0	0	4	2	0	0	4	2
	NH1	2.59	0	209	119	4.40	0	209	119
	PC1	1.31	0.06	393	1760	1.13	0.18	366	1707
	PC2	0	0	4	1	0	0	4	0
	VB1	2.05	0	101	83	7.06	1.31	97	77
	VB2	1.94	0.95	216	451	7.72	0.91	217	451
	VB3	0	0	1	3	0	0	1	3
	YC1	0.74	0	274	201	3.18	0	276	202