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Original article

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 to 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 to 5.08% among *Amblyomma americanum* populations. *Ehrlichia ewingii* prevalence was slightly higher, ranging from 0 to 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.

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 (Keirans 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 to 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 to 2012 (Nadolny et al., 2014). Because of the abundance of this tick in 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; Ewing et al., 1995). These *Ehrlichia* spp. have also been found in the American dog tick, *Dermacentor variabilis* (Say) (Murphy et al., 1998; 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 to 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.

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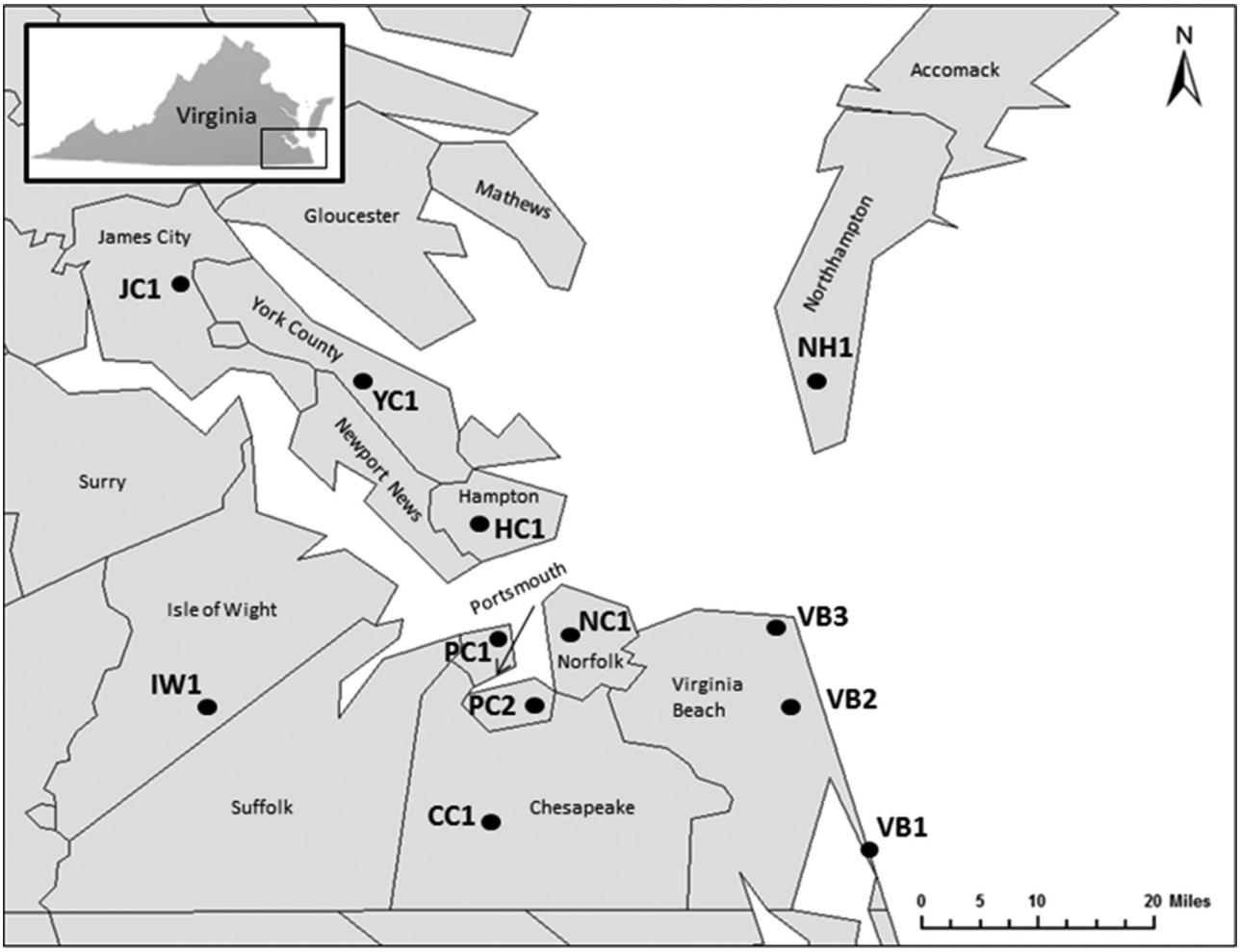

Fig. 1. Map of southeastern Virginia showing the location of the sites where ticks were collected in 2010 and 2011.

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; 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 to 4.3% (mean = 1.6 ± 1.4) in 2010 and 0 to 5.1% (mean = 1.1 ± 1.6) in 2011. Prevalence of *E. ewingii* in adults also varied by location, ranging from 0 to 8.2% (mean = 3.1 ± 2.6) in 2010 and 0 to 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