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Scientific Note

Ixodes affinis (Acari: Ixodidae) in southeastern Virginia and implications for the spread of *Borrelia burgdorferi,* the agent of Lyme disease

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Ixodes affinis Neumann is a hard-bodied (ixodid) tick known to be a competent vector for *Borrelia burgdorferi*, the agent of Lyme disease, and agents of other human diseases (Keirans et al. 1999). *Ixodes affinis* has been reported in Florida, Georgia, and South Carolina and throughout coastal North Carolina (Clark et al. 1998, Harrison et al. 2010). Harrison et al. (2010) indicated that *I. affinis* was established throughout the coastal plain of North Carolina up to the Virginia border and suggested that *I. affinis* might occur in Virginia.

Ixodes affinis is morphologically very similar to *Ixodes scapularis* Say, the primary vector for *B. burgdorferi* in the eastern United States. Although all active stages of *I. affinis* can be morphologically distinguished from *I. scapularis* (Durden and Keirans 1996, Keirans and Clifford 1978, Oliver et al. 1987), field-collected specimens are often damaged and difficult to identify. The distributions of these *Ixodes* spp. overlap in some areas, which can make visual distinction very difficult, particularly in juvenile stages (Keirans et al. 1996). Understanding the role of the two species in disease vector ecology provides an incentive to confirm the identity of any *Ixodes* species ticks in areas where ranges overlap (Goddard 1992). Traditionally *I. affinis* adults are reported to actively quest during the hot summer months when *I. scapularis* adults are dormant (Harrison et al. 2010). *I. affinis* are not known to bite humans, but are considered more important than the human biting vector, *I. scapularis*, in maintaining the enzootic spirochete cycle among mammalian host species (Oliver 1996, Oliver et al. 2003). *I. affinis* feed on many common species of mammals and as such may amplify the prevalence of *B. burgdorferi* and *B. bissettii* in these reservoir hosts (Clark 2004). Potentially this would increase the number of infected animals that in turn could transmit the pathogen to *I. scapularis*.

As part of an ongoing study to document the presence and abundance of tick vectors of infectious diseases in southeastern Virginia, individual *Ixodes* spp. ticks were identified by morphological criteria and confirmed using molecular methods. This paper presents evidence of established populations of *I. affinis* in Virginia and discusses potential implications for the spread of *B. burgdorferi* infections in mammalian host reservoirs.

Adult and nymphal ticks were collected from nine sites throughout southeastern Virginia using flags made of white denim attached to dowel rods and dragged through vegetation. The sites sampled represent a variety of habitat types, including forests and grasslands, from the Atlantic Ocean beaches to James City County, VA (Figure 1). All ticks found on flags were removed with forceps and placed in vials labeled with the time and date of collection, as well as the sampling location, temperature, and weather. Ticks were identified to the lowest taxonomic level possible using morphological criteria (Keirans and Clifford, 1978) before being frozen at -80° C. In order to ensure accurate identification of the *Ixodes* spp., molecular techniques were employed.

Ixodes ticks collected from the same site on the same day were pooled into groups of up to four adults and eight nymphs. DNA was extracted using the DNeasy Blood and Tissue Kit (Qiagen Inc., Valencia, CA) according to the manufacturer's protocol and stored at -20° C until processing. Samples were originally pooled to test for the presence of *B. burgdorferi* and other pathogens. Because multiple *Ixodes* species were not expected in the region, all pools were sequenced to determine species only after one pool suggested the presence of *I. affinis*: subsequently two individual ticks were sequenced. Our continued sampling efforts will sequence ticks individually that cannot be morphologically identified to species level. Species identification was confirmed by sequencing. A 454 bp fragment of the tick mitochondrial 16S ribosomal RNA gene was amplified on an iCycler (BioRad Laboratories, Hercules, CA) using primers 16S-1 (5'-GTCTGAACTCAGATCAAGT-3') (Macaluso et al. 2003) and 16S+1 (5'-CTGCTCAATGATTTTTTAAATTGCTGT-3'). All PCR products for sequencing were purified by using Wizard PCR Preps DNA Purification System (Promega, Madison, WI), and sequencing reactions were performed by using the BigDye Terminator v.3.1 Cycle Sequencing Kit (Applied Biosystems, Foster City, CA) as described by the manufacturer and using 16S primers. Sequence similarities were identified by a BLAST search (http://blast.ncbi.nlm.nih.gov).

Tick species identification

All *Ixodes* ticks collected were tentatively identified morphologically using microscopic examination before pooling; molecular methods were used to confirm species. A total of 94 *Ixodes* spp. ticks were collected during the summer of 2010. DNA was extracted and a fragment of the 16S rRNA was amplified from each pool. Sequencing analysis revealed that of the 55 *Ixodes* pools tested, 23 pools shared maximum identities with the *I. scapularis* 16S ribosomal RNA gene while 32 pools, including four

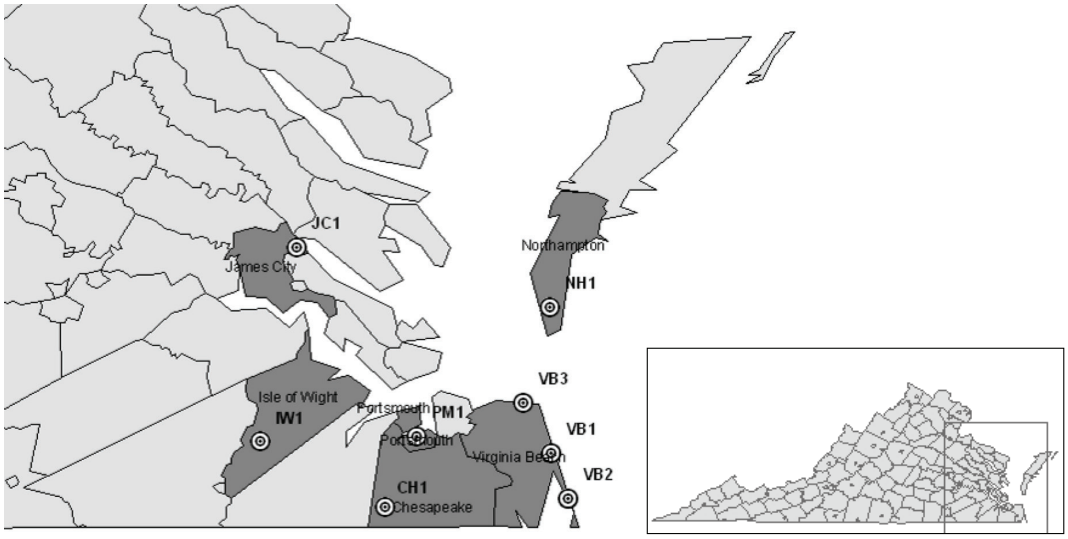

Figure 1. Map of southeastern Virginia, with counties (dark shading) and independent cities where *I. affinis* was collected using flags during the summer of 2010. Collection sites are shown and labeled according to county/independent city: JC1 – James City County; IW1 – Isle of Wight County; CH1 – City of Chesapeake; PM1 – City of Portsmouth; NH1 – Northampton County; VB1, VB2, VB3 – City of Virginia Beach sites 1, 2 and 3.

nymphal pools, shared maximum identities with the *I. affinis* ribosomal RNA gene. Maximum identities ranged from 93% to 100% for *I. affinis* and *I. scapularis*. In addition to pooled samples, two individual *I. affinis* were sequenced and a BLAST search revealed a 97% maximum identity to *Ixodes affinis* 16S ribosomal RNA gene (accession number U95879.1). An alignment showed these individually sequenced samples to be 99.8% identical to the consensus sequence of the *I. affinis* pools.

Geographic and temporal occurrence of *I. affinis* collections

Twenty-eight adult and four nymphal tick pools confirmed as *I. affinis* were collected during the summer of 2010 from eight of the sampled sites representing six independent cities and counties in southeastern Virginia (Figure 1). Ticks from a variety of habitats were collected from three sites in the city of Virginia Beach, and one site each in the city of Chesapeake, city of Portsmouth, Northampton County, James City County and Isle of Wight County (Table 1). *I. affinis* specimens were collected between April and November, 2010, with the greatest number between mid-May and mid-June (Figure 2). There was considerable overlap in the times of year that both species were active with the exception of late August. To determine temporal distribution of *I. affinis*, all sites were pooled. While it is known that tick seasonal dynamics vary with habitat type, we believe that pooling the sites in this way provides a broad picture of *I. affinis* activity in the Hampton Roads region. Although *I. affinis* was collected from sites representing grassland and numerous forest types, our most productive collection site bordered the Great Dismal Swamp in Chesapeake and represented an old-field habitat undergoing secondary succession.

Our results confirm the suggestion by Harrison et al. (2010) that *I. affinis* has extended its range north beyond the North Carolina border and into the coastal plain of southeastern Virginia. The discovery of both adults and nymphs indicates that breeding populations of *I. affinis* are now established in Virginia. *Ixodes affinis* was uncommon in North Carolina prior to 1988 (Harrison et al. 2010), which suggests a recent Northward expansion into Virginia only in the last two decades. It is plausible that *I. affinis* crossed from North Carolina to Virginia through one of the numerous potential hosts that utilize coastal plain habitats in both states (Webster et al. 1985). Two important hosts linked to *I. affinis* abundance in Georgia and South Carolina are the cotton mouse and cotton rat (Durden and Oliver 1999, Clark et al. 2001, Oliver et al. 2003). These and related species are abundant in southeastern Virginia and are common at the Chesapeake site where 11 of the 12 pools

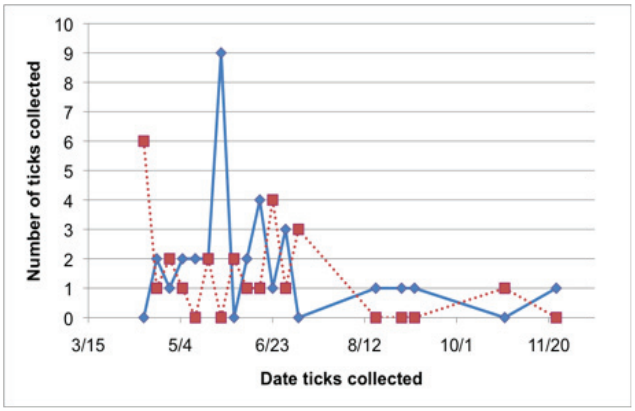

Figure 2. Total number of *I. affinis* (solid line, blue diamonds) and *I. scapularis* (dotted line, red squares) collected from all sites in southeastern Virginia from March through November, 2010. This graph assumes that all ticks in a given pool are of the same species.

Table 1. Results of sequencing tests for *Ixodes* spp. ticks collected using flags at each study site over the summer of 2010. All ticks were adults except as noted at one site. Basic habitat information for each site is also provided. See Figure 1 for identification and location of each site.

Site	**CH1**	**IW1**	**JC1**	**NH1**	**PM1 (Adults)**	**PM1 (Nymphs)**	**VB1**	**VB2**	**VB3**	**Total**
Habitat Type	Successional old field (grassland)	Mixed pine-deciduous forest	Deciduous forest	Successional old field (grassland)	Mixed pine-deciduous forest	Mixed pine-deciduous forest	Deciduous forest	Dunes/scrub forest	Deciduous forest	
Pools with *I. affinis* present	11	5	3	1	0	4	6	1	1	32
Total *Ixodes* spp. pools	12	12	3	1	3	14	7	2	1	55
Total *Ixodes* spp. ticks	18	15	3	2	3	32	13	6	2	94

of adult ticks contained at least one *I. affinis*.

The discovery of *I. affinis* in Virginia has implications for the disease vector ecology of ticks in the genus *Ixodes* in Virginia. Summer time activity of *I. affinis* adults has been a key behavioral trait that differentiates them from *I. scapularis*, which has been reported to quest more actively in the cooler months (Clark et al. 1998, Goddard 1992). While our report found that *I. affinis* peak in mid-May to mid-June, the continued questing activity of *I. affinis* until November and the persistent activity of *I. scapularis* in late spring and early summer complicates the distinction of species by their seasonal questing behaviors. Because of the difficulty of differentiating between *I. affinis* and *I. scapularis* relying on morphology alone, it is possible that *I. affinis* is more widespread in Virginia than we report. The northward expansion of this tick throughout the coastal plain of the southeastern United States suggests that areas further north that share coastal plain characteristics may also be vulnerable to invasion by this tick.

Although *I. affinis* rarely bites humans and thus plays little if any role in direct transmission of pathogens to humans, *I. affinis* has been reported to be more important than *I. scapularis* in maintaining the enzootic cycles of *B. burgdorferi* and *B. bissettii* (Harrison et al. 2010, Maggi et al. 2010, Oliver 1996, Oliver et al. 2003) in the southeastern United States. The discovery of established populations of *I. affinis* along with *I. scapularis* in areas of Virginia with many competent host species could have implications for the amplification of *B. burgdorferi* and *B. bissettii* cycles in wild animal hosts. Continued monitoring of the tick populations is needed to determine what effect a second competent *B. burgdorferi* vector may have on Lyme borreliosis in southeastern Virginia.

This paper serves to document widespread populations of *I. affinis* in a variety of habitats throughout southeastern Virginia. Adult and nymph *I. affinis* in Virginia appear to be actively questing throughout spring, summer, and autumn in this region. These populations were discovered through comprehensive surveys of tick populations at over 15 sites in southeastern Virginia. As we continue to collects ticks from around this region, *Ixodes* ticks will be individually sequenced if necessary for identification to species. Continuations of our sampling efforts in this region will provide us with more information about the ecology of *I. affinis* at the northern extent of its range, including which habitats support the most abundant adult and nymph populations and how habitat impacts life history parameters and interactions with *I. scapularis*.

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REFERENCES CITED

Clark, K. 2004. *Borrelia* species in host-seeking ticks and small mammals in northern Florida. J. Clin. Microbiol. 42: 5076 – 5086.

Clark, K.L., J.H. Oliver, Jr., D.B. McKechnie, and D.C. Williams. 1998. Distribution, abundance, and seasonal activities of ticks collected from rodents and vegetation in South Carolina. J. Vector Ecol. 23: 89-105.

Clark, K.L., J.H. Oliver, Jr., J.M. Grego, A.M. James, L.A. Durden, and C.W. Banks. 2001. Host associations of ticks parasitizing rodents at *Borrelia burgdorferi* enzootic sites in South Carolina. J. Parasitol. 87: 1379-1386.

Durden, L.A. and J.H. Oliver, Jr. 1999. Ecology of *Ixodes scapularis* and Lyme disease in coastal Georgia. In*:* G.R. Needham, R. Mitchell, D.J. Horn, and W.C. Welbourn (eds.) pp. 379-383. *Acarology IX*: Volume 2, Symposia. xvii + 507 pp. Ohio Biological Survey, Columbus, Ohio.

Durden, L.A. and J.E. Keirans. 1996. Nymphs of the genus *Ixodes* (Acari: Ixodidae) of the United States: taxonomy, identification key, distribution, hosts, and medical/veterinary importance. Thomas Say Publications in Entomology: Monographs. Entomological Society of America, Lanham, MD.

Goddard J. 1992. Ecological studies of adult *Ixodes scapularis* in central Mississippi: Questing activity in relation to time of year, vegetation type, and meteorologic conditions. J. Med. Entomol. 29: 501-506.

Harrison, B.A., W.H. Rayburn Jr., M. Toliver, E.E. Powell, B.R. Engber, L.A. Durden, R.G. Robbins, B.F. Prendergast, and P.B. Whitt. 2010. Recent discovery of widespread *Ixodes affinis* (Acari: Ixodidae) distribution in North Carolina with implications for Lyme disease studies. J. Vector Ecol. 25: 174-179.

Keirans, J.E and C. M. Clifford. 1978. The genus *Ixodes* in the United States: a scanning electron microscope study and key to the adults. J. Med. Entomol. Suppl. 2: 1-149.

Keirans, J.E., H.J. Hutcheson, L.A. Durden, and J.S.H. Klompen. 1996. *Ixodes* (*Ixodes*) *scapularis* (Acari: Ixodidae): Redescription of all active stages, distribution, hosts, geographical variation, and medical and veterinary importance. J. Med. Entomol. 33: 297-318.

Keirans, J.E., G.R. Needham, and J.H. Oliver, Jr. 1999. The *Ixodes ricinus* complex worldwide: Diagnosis of the species in the complex, hosts and distribution. In: G.R. Needham, R. Mitchell, D.J. Horn, and W.C. Welbourn (eds.) pp. 341- 347. Acarology IX: Volume 2, Symposia. xvii + 507 pp. Ohio Biological Survey, Columbus, Ohio.

Macaluso, K.R., A. Mulenga, J.A. Simser, and A.F. Azad. 2003. Differential expression of genes in uninfected and *Rickettsia*-infected *Dermacentor variabilis* ticks as assessed by differential-display PCR. Infect. Immun. 71: 6175-6170.

Maggi, R.G., S. Reichelt, M. Tolliver, and B. Engber. 2010. *Borrelia* species in *Ixodes affinis* and *Ixodes scapularis* ticks collected from the coastal plain of North Carolina. Ticks Tick-borne Dis. 1: 168-171.

Oliver, J.H. Jr. 1996. Lyme borreliosis in the southern United States: A review. J. Parasitol. 82: 926-935

Oliver, J.H. Jr., J.E. Keirans, D.R. Lavender, and H.J. Hutcheson. 1987. *Ixodes affinis* Neumann (Acari: Ixodidae): new host and distribution records, description of immatures, seasonal activities in Georgia, and laboratory rearing. J. Parasitol. 73: 646-652.

Oliver, J.H. Jr., T. Lin, L. Gao, K.L. Clark, C.W. Banks, L.A. Durden, A.M. James, and F.W. Chandler, Jr. 2003. An enzootic transmission cycle of Lyme borreliosis spirochetes in the southeastern United States. Proc. Nat. Acad. Sci. U.S.A. 100: 11642-11645.

Webster, W.D., J.E. Parnell, and W.C. Biggs, Jr. 1985. *Mammals of the Carolinas, Virginia, and Maryland.* Univ. North Carolina Press, Chapel Hill, NC. 255 pp.