Ticks and Tick-borne Diseases xxx (xxxx) xxx–xxx

Contents lists available at ScienceDirect

Ticks and Tick-borne Diseases

journal homepage: www.elsevier.com/locate/ttbdis

Research paper

Natural history of *Ixodes affinis* in Virginia

Robyn M. Nadolny[a,b,*], Holly D. Gaff[a,c]

[a] Old Dominion University, Department of Biological Sciences, Norfolk, VA, USA
[b] U.S. Army Public Health Center Tick-Borne Disease Laboratory, Aberdeen Proving Ground, MD, USA
[c] University of KwaZulu-Natal, School of Mathematics, Statistics, and Computer Sciences, Durban, South Africa

ARTICLE INFO

Keywords:
Ixodes affinis
Invasions
Range expansions
Ticks
Tick-host interactions

ABSTRACT

The ixodid tick species *Ixodes affinis* is expanding its range northward, changing the tick community population dynamics in the Mid-Atlantic United States. We present five years of surveillance on newly established populations of *I. affinis* throughout southeastern Virginia and discuss the habitat and host associations of *I. affinis* in this northernmost extent of its range. We found that *I. affinis* populations tend to persist once they are established, and populations tend to increase as ecological succession progresses, provided a vegetated understory persists. Populations of *I. affinis* were never found in the smallest habitat fragments or in xeric dune habitats, and the highest densities of *I. affinis* were found in mixed pine-hardwood forests with an herbaceous understory. We also document several new mammalian hosts for *I. affinis,* including house mice (*Mus musculus*) and coyotes (*Canis latrans*) and discuss how these hosts may facilitate the continued dispersal of *I. affinis* and the maintenance of these newly established populations.

1. Introduction

Ixodes affinis is a non-human-biting hard tick known for its role in the sylvatic cycle of *Borrelia burgdorferi* in the southeastern United States (Oliver et al., 2003; Maggi et al., 2010; Rudenko et al., 2013). *Ixodes affinis* is native to Central and South America as far south as Brazil, with the first U. S. specimens collected in Florida in 1953 (Kohls and Rogers, 1953). *Ixodes affinis* has since expanded its range through Florida, Georgia, and South Carolina, with new populations discovered in North Carolina and Virginia in the last decade (Harrison et al., 2010; Nadolny et al., 2011). Genetic evidence supports the hypothesis that the populations in North Carolina and Virginia are more recently established than those in more southern states, including South Carolina and Georgia, and that the northern and southern ticks represent two distinct populations with a clear geographic break along the border between North and South Carolina (Nadolny et al., 2015). North Carolina and Virginia ticks are closely related in a single genetic clade based on 16S mitochondrial haplotype data. In order to create the high connectivity observed in the North Carolina and Virginia northern population, these ticks are likely being dispersed as adults via mammalian hosts (Nadolny et al., 2015).

Ixodes affinis is known to parasitize 15 mammal and seven bird species in the United States and Canada, and has been collected from habitats across the piedmont, sandhills, and coastal plain ecoregions of North and South Carolina, as well as in forested and scrub habitats in Florida and Georgia (Clark et al., 2001; Clark, 2004; Nelder and Reeves, 2005; Harrison et al., 2010; Heller et al., 2016). At the northernmost extent of its range in Virginia, *I. affinis* has been collected from many habitats throughout the coastal plain (Nadolny et al., 2011). When they are present, these ticks are generally found in low densities in riparian habitats along the coastal plain (Clark, 2004; Nadolny et al., 2011). The possibility of further northward expansion to other coastal plain habitats with appropriate host communities cannot be discounted.

To understand how *I. affinis* will influence the existing community of ticks and pathogens in northern states, it is important to recognize any differences in life history characteristics this tick may be exhibiting at the northern extent of its range, such as altered associations with habitats and hosts, or altered phenology. One example of altered phenology influencing pathogen dynamics is the synchrony of questing *Ixodes scapularis* life stages observed in the Midwest but not the Northeastern United States (Stromdahl et al., 2014). Synchronous feedings influenced by colder climates may enhance maintenance of short-lived strains of *B. burgdorferi*, and may result in greater prevalence rates of other pathogens such as *Anaplasma phagocytophilum* and *Babesia microti* (Gatewood et al., 2009; Stromdahl et al., 2014). Such differences in *I. affinis* questing patterns or host choice could have implications for the sylvatic cycle of *B. burgdorferi*, and for human health in areas where both *I. affinis* and the human-biting *I. scapularis* are abundant. This study presents the results of five years (2010–2014) of field surveillance on *I. affinis* populations in southeastern Virginia and commentary on

* Corresponding author at: U.S. Army Public Health Center Tick-Borne Disease Laboratory, Aberdeen Proving Ground, MD, USA.
E-mail address: robyn.m.nadolny.civ@mail.mil (R.M. Nadolny).

http://dx.doi.org/10.1016/j.ttbdis.2017.09.016
Received 3 October 2016; Received in revised form 13 September 2017; Accepted 28 September 2017
1877-959X/ Published by Elsevier GmbH.

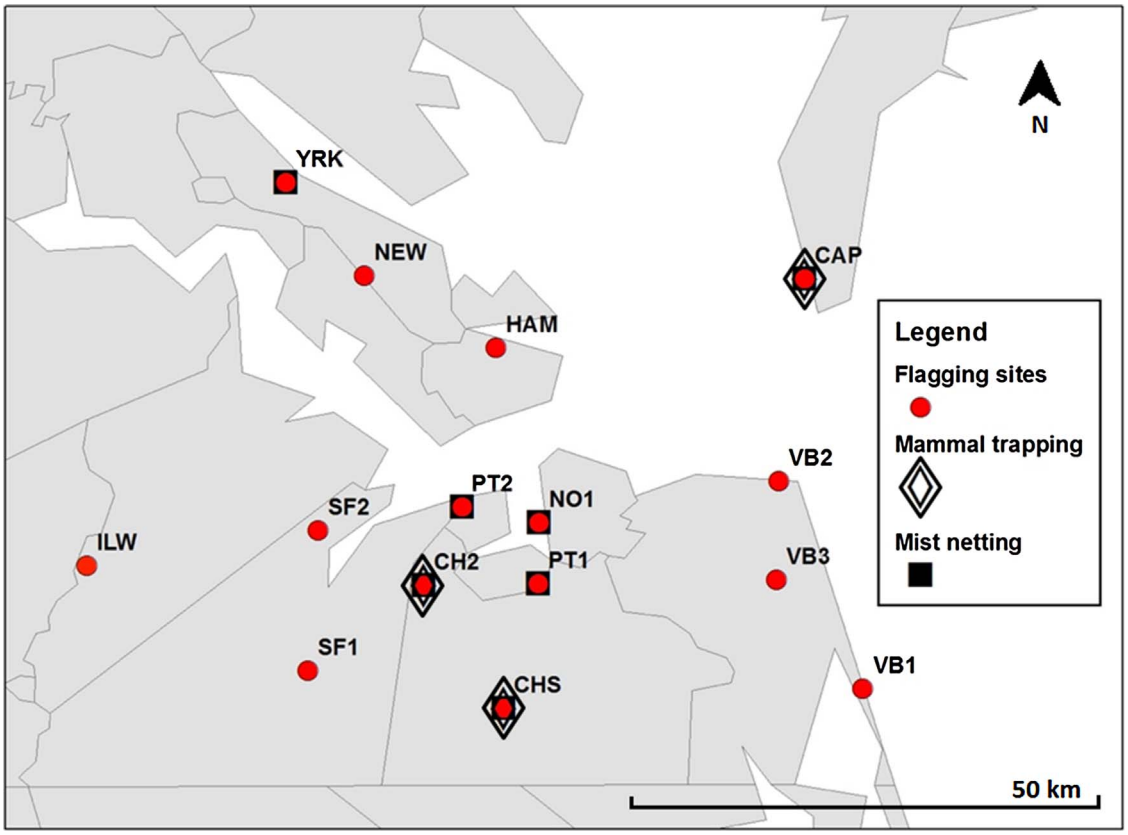

Fig. 1. Map of *Ixodes affinis* collection sites, 2010–2014. Map shows southeastern Virginia counties and independent cities, with circles marking sites that were flagged weekly or bi-weekly from 2010 to 2014. For years of sampling and brief habitat descriptions of these sites, see Table 1. Sites where mist netting also took place are surrounded by a square, and sites where small mammal trapping also took place are marked with a diamond.

the influence of ecological factors including host use, tick phenology, and habitat associations on the northward range expansion of this tick.

2. Materials and methods

2.1. Site selection, habitat descriptions, and collection of questing ticks

Questing adult *I. affinis* were collected from public and privately owned lands throughout southeastern Virginia from 2010 to 2014. Fifteen sites representing a variety of habitats were sampled weekly during the summer months and at least monthly throughout the year (Fig. 1, see Table 1 and Table S1 for habitat information). Sites were selected prior to the discovery of *I. affinis* in the region (Nadolny et al., 2011) as part of a larger tick surveillance program in southeastern Virginia, to include a broad selection of habitats typical to the peri-urban Mid-Atlantic and southeastern United States. Habitats sampled included fields in various stages of succession to forest, as well as established deciduous forests, and forests dominated by loblolly and longleaf pines (Tables 1 and S1). Each site comprised one or more transects designed to sample habitats representative of the site. All sites were located in the coastal plain, in Middle Atlantic coastal forest habitat (Olson et al., 2001).

All transects were marked with surveyors flags so that the same transect was walked and flagged during each sampling event. In order to determine the area sampled at each site, GPS coordinates were taken along each transect. ArcGIS 9.3 (www.esri.com) was used to draw a line connecting the GPS points from each transect and to draw a 2 m buffer around the line. Ticks were collected using 1-m^2 white denim flags attached to wooden dowel rods swept over the ground and through low vegetation as previously described (Ginsberg and Ewing, 1989). Flags were checked for ticks every few meters, and careful training was provided to technicians to ensure consistency of collection. Between one and three technicians collected at each site, and the number of technicians was incorporated into the denominator when determining tick density. All ticks found clinging to the flag material were removed from the flag with forceps and placed in vials labeled with the time and date of collection, as well as the sampling location, temperature, and weather data. Ticks were frozen at −20 °C until processed, morphologically identified (Keirans and Clifford, 1978; Oliver et al., 1987; Durden and Keirans, 1996), and then stored frozen at −80 °C and held for future molecular processing.

2.2. Collection of ticks from hosts

Host-targeted sampling techniques were employed throughout 2010–2014, including collection of ticks from large mammals at hunt check locations, collaboration with veterinarians to collect ticks from companion animals, serendipitous sampling of road-killed mammals, small mammal live-trapping, avian sampling using mist-nets, and opportunistic hand-capture of reptiles (ODU IACUC # 11-012, 13-018, 13-008). In addition, ticks found crawling on or attached to humans throughout southeastern Virginia were submitted periodically to the Old Dominion University Tick Research Team between 2011 and 2014 as a result of local outreach and educational programs.

Large wild mammals were sampled for ticks through collaborations with local hunters, game managers, and state biologists, and through checking road-killed animals for ticks. Any ticks found on dead mammals were removed with forceps, placed in vials, and returned to the laboratory where they were frozen at −20 °C until processed. Domestic animals were sampled through collaborations with veterinarians with practices in southeastern Virginia. If any ticks were found on an animal patient, ticks were removed, labeled, and frozen until they could be