Amblyomma americanum and *Ixodes scapularis* (Acari: Ixodidae) within the Capital Beltway and Associated Human Pathogens in Greenbelt National Park, Maryland (USA)[1]

J.F. Carroll[2], M. Miller[3], and M. Kramer[4]

U.S. Department of Agriculture, Agricultural Research Service (USDA, ARS), Invasive Insect Biocontrol and Behavior Laboratory, Beltsville Agricultural Research Center, Beltsville, Maryland 20705, USA.

J. Entomol. Sci. 55(2): 252–261 (April 2020)

Abstract Populations of host-seeking blacklegged tick, *Ixodes scapularis* (Say) and lone star tick, *Amblyomma americanum* (L.), nymphs were monitored at selected sites for 4 yr and at additional randomly selected sites in the Greenbelt National Park, MD for two of those years. Ticks collected from the random sites during the second year of the study were tested for the presence of human pathogens. *Borrelia burgdorferi* Johnson et al. was detected in 22.2% of the *I. scapularis* nymphs collected while *Anaplasma phagocytophilum* Foggie (Dumler et al.) was detected in 3.7%, and one nymph was coinfected with both pathogens. No *I. scapularis* nymphs tested positive for *Babesia microti* (França) and no *A. americanum* nymphs tested positive for *Ehrlichia* spp. In the years when both random and nonrandom sites were sampled (sampled $\leq$2 d apart, $n = 14$ d), significantly more *A. americanum* nymphs ($P = 0.003$) were captured at the nonrandom sites than at the random sites; no difference ($P = 0.2415$) was found for *I. scapularis* nymphs. No density effect due to vegetational communities was found for nymphs of either species of tick. Host-seeking nymphs of both species of ticks were abundant the first year of flag sampling, dropped dramatically in numbers the second year, and gradually increased (particularly *A. americanum*) the following 2 yr. The annual variations in tick densities demonstrate the value of early season–monitoring of tick populations on park premises, which affords park managers an opportunity to take appropriate measures in the event of a year of high tick abundance.

Key Words *Borrelia burgdorferi*, *Ehrlichia phagocytophilum*, population fluctuation

Every year, millions of visitors patronize national, state, and municipal parks for a variety of recreational purposes. Because parks typically feature at least some natural habitat and associated vertebrate and invertebrate fauna, exposure to biting arthropods can and should be expected by visitors. At elevated risk are visitors who are unfamiliar with the arthropod hazards of a particular park, especially one with camp grounds. Out-of-state visitors often arrive from areas of the country where ticks and tick-borne disease are not a concern and are consequently naïve to the dangers of exposure to ticks. Greenbelt National Park (445.2 ha) is not only located

[1]Received 11 May 2019; accepted for publication 26 June 2019.
[2]Corresponding author (retired) (email: eppiecarroll@gmail.com),4002 September Song Drive, Manchaca, TX 78652, USA.
[3]Laboratory Sciences Division, Public Health Command-Atlantic, Fort Meade, MD 20755-5225, USA.
[4]USDA, ARS, Statistics Group, Beltsville Agricultural Research Center, Beltsville, MD 20705, USA.

near two large urban centers (Washington, DC and Baltimore, MD), but many campers use the park as a home base while sightseeing in and around the nation's capital just a few kilometers from the park.

By their nature, parks are ideal locales for human-tick contact and have been the sites for several research studies as tick problems have grown in North America and elsewhere (Eisen et al. 2013; Paskewitz et al. 2001; Prusinski et al. 2014). Recently, Johnson et al. (2016) reported infection rates of *Borrelia burgdorferi* Johnson et al., *B. miyamotoi* Fukunaga et al., *Anaplasma phagocytophilum* Foggie (Dumler et al.), and *Babesia microti* (França) in nymphal *Ixodes scapularis* Say collected in one New England and eight Mid-Atlantic National Parks including Rock Creek Park in Washington, DC, Manassas National Battlefield and Prince William Forest Park in nearby Virginia, and Catoctin Mountain Park and Monocacy National Battlefield in central Maryland.

Lone star ticks, *Amblyomma americanum* (L.), have long been notorious as nuisance biters in parks and other venues (Barnard et al. 1988). There are growing concerns about the role of this species in the transmission of pathogens to humans and about its expanding range (Childs and Paddock 2003; Goddard and Varela-Stokes 2009; Jordan and Egizi 2019; Stromdahl and Hickling 2012). The aggressive, host-seeking behavior of *A. americanum* and its capacity to attain dense populations make it readily noticed by humans (Armstrong et al. 2001). Lyme disease is major human health problem in parts of the United States (Centers for Disease Control [CDC] 2017). The blacklegged tick, *I. scapularis*, the principal vector of *B. burgdorferi*, the causative agent of Lyme disease (Burgdorfer et al. 1982; Spielman et al. 1985), became widespread in central Maryland in the 1980s and 1990s, with Maryland attaining a high incidence of Lyme disease (Pepin et al. 2012); it is regularly among the top 10 states for Lyme disease in the United States (CDC 2017).

In 2008, we were alerted to tick problems on a school campus bordered on three sides by Greenbelt National Park. In order to better understand the tick situation on the school premises, we obtained approval from the National Park Service, National Capital Parks–East to sample for host-seeking ticks in Greenbelt National Park. The disturbingly high densities of *A. americanum* and *I. scapularis* populations we found by flagging in the park in 2009 led us to broaden the sampling and conduct an infectivity study in 2010, with continued population sampling in 2011 and 2012.

Materials and Methods

Study area and sample sites. Greenbelt National Park is in Prince George's Co., MD, and lies northeast of Washington, DC within the Capital Beltway. The park straddles the Baltimore–Washington Parkway (Gladys Noon-Spellman Parkway) (a roughly northeast–southwest highway). Human use of the park is mostly confined to the area west of the parkway, as there are no campgrounds, picnic areas, ball fields, or trails in Greenbelt National Park east of the parkway. The park is mostly wooded and harbors white-tailed deer, *Odocoileus virginianus* (Zimmermann) and smaller vertebrates essential to sustaining tick populations.

In 2009, 17 nonrandom sites were selected by on-site inspection for repeated sampling. Only wooded sites that appeared capable of supporting *I. scapularis* and

A. americanum (Ginsberg and Ewing 1989; Lubelczyk et al. 2004; Ostfeld et al. 1995; Sonenshine 1993) were considered for sampling; parking lots, closely mowed ball fields, and road sides were not included. Other factors in nonrandom site selection were accessibility from roads, proximity to camp grounds and trails, and geographical representation of the north, center, and south of the area west of the parkway. The nonrandom sites were sampled annually 2009–2012. In 2010, 51 sets of geographic coordinates were randomly selected and each designated the center of a sample site. Coordinates of the random sample sites were transposed onto a map of the vegetational communities in Greenbelt National Park, and each site was categorized as to the vegetational community at its location. Four vegetational communities (low elevation mixed oak/heath, mesic mixed hardwood, pine oak woodland, and successional mixed deciduous forest) accounted for 84% of the random sites, with low elevation mixed oak/heath (22 sites) and mesic hardwood (9 sites) the most frequently represented. The portion of the park east of the Baltimore–Washington Parkway constitutes almost one third of the park's total area. Because of its disproportionately low use by the public and its inaccessibility (no trails), only seven of the 51 random sites were located east of the parkway. In 2010, all 51 random sites were sampled but, in 2011, the number of random sites sampled was reduced to 20. This study was conducted under a research permit (NACE-2010-SCI-0017) from the U.S. Department of Interior, National Park Service, National Capital Parks–East.

Sampling tick populations and collection of ticks for pathogen analysis. At each sampling site, a 0.5×0.5-m flag of laminated flannel crib cloth was flip-flopped on leaf litter and low vegetation as the operator walked slowly for 30 s, advancing ~10 m in a straight line, as obstructions allowed. This subsample was repeated 10 times at each of the sample sites on each sample date. Ticks on the cloth at the end of the subsample were identified, counted, and returned to the route just flagged. On three dates from late May–July 2009, each of 17 nonrandom sites in the park was sampled for host-seeking ticks, except for the last date in July when 10 sites were sampled. Sampling was expanded in 2010 to include 51 random sites sampled in addition to the 17 nonrandom sites. As in 2009, all the 2010 sites were sampled on three dates from May–July, which is the peak period of host-seeking activity for nymphal *I. scapularis* and nymphal and adult *A. americanum*. In 2010, after counting the ticks on the flag, a maximum of one *I. scapularis* nymph and three *A. americanum* nymphs captured on each sample date at each random sample site were placed in plastic, snap-cap vials so that 100 *I. scapularis* nymphs and 300 *A. americanum* nymphs were collected for pathogen analysis. An additional four nymphal, nine female, and eight male *A. americanum* captured at the sample sites were included in the pathogen testing. The remainder of the ticks caught on the flags were identified and returned to the route just flagged. The original 17 nonrandom sites were sampled on three dates during May–July 2011 (except six sites which were sampled only on the first two dates), as were 20 of the random sites, and the ticks were returned to the route just flagged. In 2012, the 17 nonrandom sites were similarly sampled on three dates during May–July.

Pathogen analysis. Captured ticks retained for pathogen analysis were placed in plastic, snap-cap vials labeled as to sample site and brought to the laboratory where the ticks were transferred to glass vials containing 95% ethanol. Adult and nymphal ticks were identified and placed individually in 500 μl of Tissue Lysis Buffer

(Qiagen, Valencia, CA). Ticks were bisected with a sterile knife and incubated with the addition of proteinase K prior to nucleic acid purification according to kit directions with the DNeasy Blood and Tissue Kit (Qiagen). Purified nucleic acids were eluted with 100 μl of elution buffer. The bisected ticks, remaining lysate, and purified nucleic acids were stored at −80°C for future analysis.

Purified nucleic acid preparations from individual *I. scapularis* ticks were screened for *Borreli*a and *Anaplasma* species by a multiplex assay targeting the 235 rRNA and msp2 genes of *Borrelia* and *Anaplasma*, respectively, as previously described (Courtney et al. 2004). In addition, the samples determined positive for *Borrelia* species were further confirmed as *Borrelia burgdorferi* using real-time polymerase chain reaction (qPCR) targeting the N40.seq gene (Straubinger 2000). Likewise, *Anaplasma* species-positive samples were further tested with a qPCR singleplex targeting a 106-base pair fragment of the 16s rRNA gene (Pusteria et al. 1999). After preparation, individual *A. americanum* ticks were screened by a multiplex assay targeting the groEL gene of *Ehrlichia* species (Bell and Patel 2005). The *Borrelia* and *Anaplasma* screening qPCR assay was performed using the LightCycler® TaqMan® Master kit (Roche, Indianapolis, IN) on the Roche LightCycler® 2.0. The *Ehrlichia* screening qPCR assay was performed using the LightCycler FastStart DNA Master^{PLUS} HybProbe kit (Roche) on the Roche LightCycler 2.0. All confirmation qPCR assays were performed using the LightCycler TaqMan Master kit (Roche) on the Roche LightCycler 2.0.

Statistical methods. Analyses were conducted using linear models on transformed counts. A Box-Cox family of transformations of counts +1 (values must be >0 for a Box-Cox transformation) suggested that the log transformation was appropriate (i.e., log [counts + 1] produced homogeneous variances across the range of values). Various linear models were fit and *F*-tests used to determine significance ($P < 0.05$). Not reported are results from models used to check for spatial autocorrelation of the residuals; we found no evidence that tick abundance at any particular site was influenced by abundances at nearby sites after accounting for main effects such as tick species and date.

Results

Host-seeking nymphs of both *I. scapularis* and *A. americanum* were found at each of the 51 and 20 random sites in 2010 and 2011, respectively. Both species were also found at each of the 17 nonrandom sites sampled annually 2009–2012. On the nonrandom sites, densities of host-seeking nymphs of both species were highest in 2009, followed by a precipitous decline in 2010 (Fig. 1). By 2012, *A. americanum* numbers had risen, with some higher-density areas appearing that were not found in 2010 and 2011. Numbers of *I. scapularis* nymphs showed less of an increase than did those of *A. americanum* nymphs from 2010 to 2012.

No differences in densities of either *I. scapularis* or *A. americanum* nymphs were found among the nine vegetational communities that comprised the random sites (*F*-test on log [tick count + 1], $P = 0.2507$ for vegetational communities, df = 8, 102; $P = 0.3374$ for the species by vegetational communities interaction term, df = 8, 102). However, when counts for *A. americanum* nymphs sampled at random and nonrandom sites ≤2 d apart ($n = 14$ d) in 2010–2011 were compared, significantly

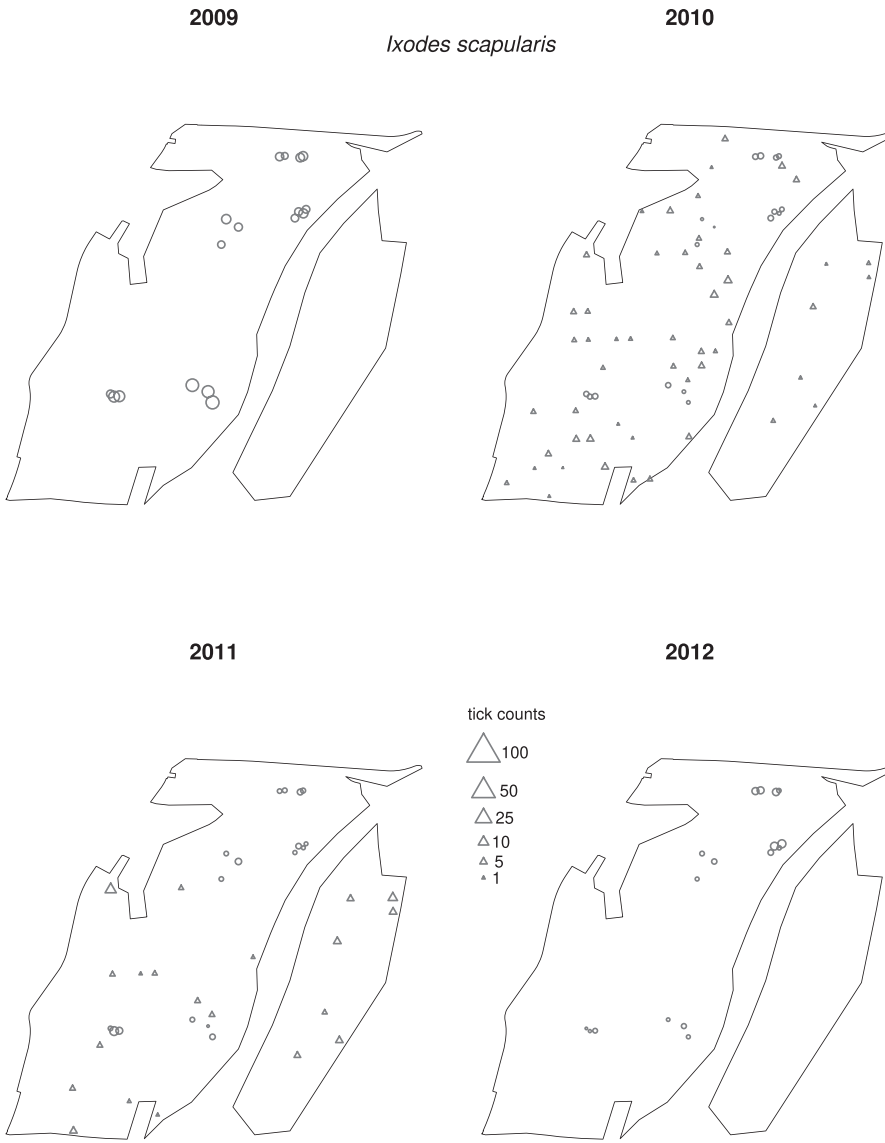

Fig. 1. Relative abundance of host-seeking nymphs of *I. scapularis* and *A. americanum* at sample sites in Greenbelt National Park 2009–2010. Both species of ticks were found at least once at every sample site. Seventeen nonrandom sites (circles) were sampled each year and 51 and 20 random sites (triangles) were sampled in 2010 and 2011, respectively. Note the decrease in size of the counts from 2009 and 2010. Symbols represent average of three samples per site each year, except six nonrandom sites which were only sampled twice in 2009 and twice in 2011.

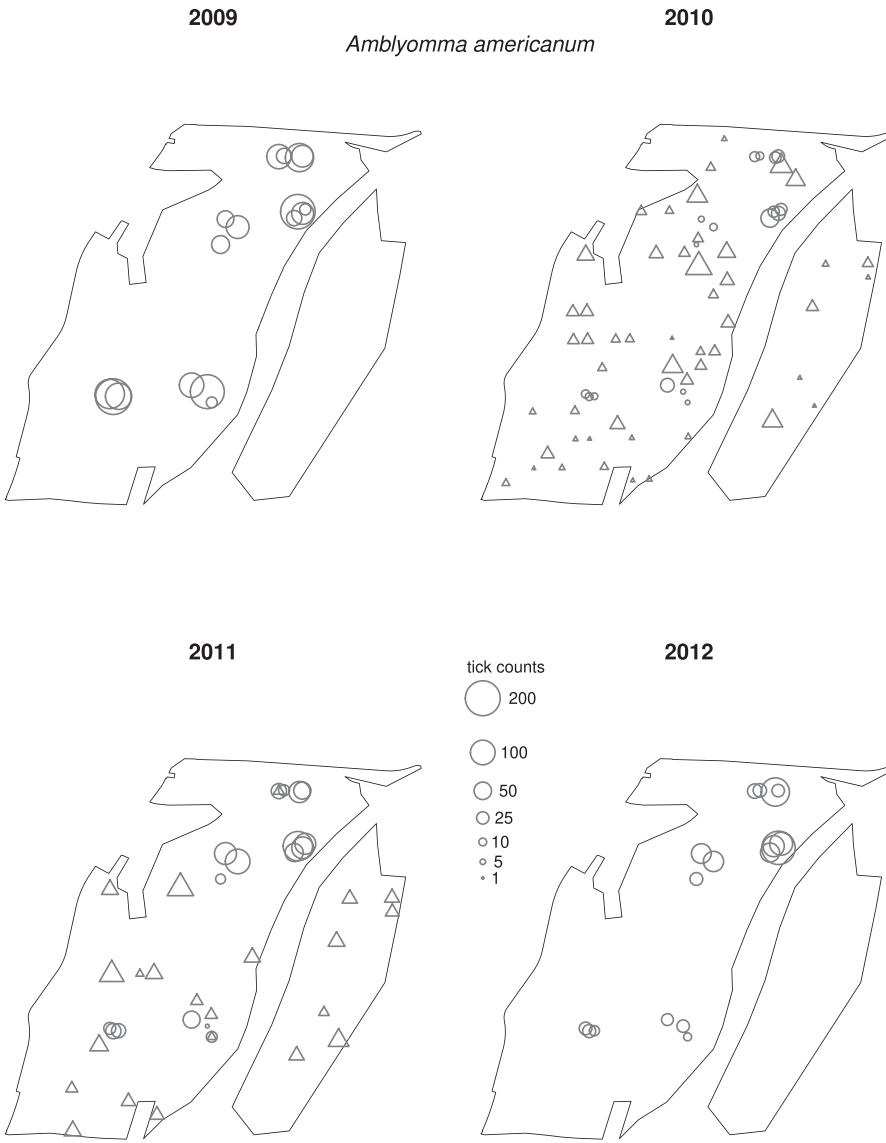

Fig. 1. Continued.

(F-test on log [tick count + 1], $P = 0.00303$, df = 1, 137) more *A. americanum* were captured at nonrandom sites than at random sites whereas on those sample dates the number of nymphs at random and nonrandom sites was similar for *I. scapularis* (F-test on log [tick count +1], $P = 0.2415$, df = 1, 137). Not unexpectedly, few adult *I. scapularis* were captured, as the sampling was conducted well after the peak of their spring host-seeking season. Adult *A. americanum* were found predominantly in

May and June and occurred at most sampling sites. In the earliest count of the year (May–June), at least one adult *A. americanum* was captured at all nonrandom sites in 2009 and at least at 82.4% of these 17 sites in 2010, 2011, and 2012. In 2010, at least one adult *A. americanum* was found at 80.4% of the random sites (mean 3.80 ± standard deviation [SD] 4.22 adults) but at 60% of the 20 random sites (mean 0.81 ± SD 0.94 adults) sampled in 2011.

Eighty-one of the *I. scapularis* nymphs collected in 2010 were tested for *B. burgdorferi* and *A. phagocytophilum*. Eighteen (22.2%) of the nymphs tested positive for *B. burgdorferi*, and three nymphs (3.7%) tested positive for *A. phagocytophilum*, with one nymph coinfected with both pathogens. No *I. scapularis* nymphs tested positive for *Babesia microti* nor did any *A. americanum* nymphs ($n =$ 304) or adults ($n = 17$) test positive for *Ehrlichia* spp.

Discussion

The precipitous decrease in the densities of the populations of host-seeking *I. scapularis* and *A. americanum* nymphs observed at Greenbelt Park from 2009 to 2010, followed by gradual annual increases in densities, is not unusual (Carroll and Schmidtmann 1996, Carroll et al. 2009a, b). We did not find any difference in densities of host-seeking nymphs of either *I. scapularis* or *A. americanum* among the nine vegetational communities represented in the random sites. Other factors may contribute in determining whether a sample site is favorable for high tick density. For instance, Van Horn et al. (2018) reported that in Missouri, densities of host-seeking *A. americanum* on slopes were related to whether the slope faced north, south, east, or west. Interestingly, at Greenbelt National Park counts of *A. americanum* nymphs were significantly higher at nonrandom sites than at random sites sampled $\leq$2 d apart ($n = 14$ d) in 2010–2011 whereas counts of *I. scapularis* nymphs did not differ between random and nonrandom sites. Only sites that appeared favorable for supporting *I. scapularis* and *A. americanum* (Ginsberg and Ewing 1989; Lubelczyk et al. 2004; Ostfeld et al. 1995; Sonenshine 1993) were chosen as nonrandom sites. We did not select nonrandom sites in preference of either *I. scapularis* or *A. americanum* but chose the sites in 2009 as an initial effort to establish whether ticks were a problem in Greenbelt National Park. To maximize the number of sites that could be sampled in the time available on a given date, the selection of nonrandom sites was biased toward ease of access (short walking distance from roads). In contrast, considerable time (much more than in actual sampling) was spent walking on and off trails to and from random sites and locating the random sites by global positioning system. With the number of personnel available to do sampling, the great amount of time expended getting to and from the random sample sites on foot made it difficult to finish the three samplings per site protocol within the host-seeking season of *A. americanum* and *I. scapularis* nymphs. Inasmuch as we found more *A. americanum* nymphs at the nonrandom sites than at random sites sampled $\leq$2 d apart, further investigation comparing tick counts from random and nonrandom sample sites would be of value.

Infection prevalence (proportion) among *I. scapularis* nymphs in Greenbelt Park (0.22) was similar to the nymphal infection prevalences (0.10–0.36) reported by Feldman et al. (2015) for various residential locations in Maryland and by Johnson

et al. (2016) for Catoctin Mountain Park and Monocacy National Battlefield in Maryland, Rock Creek Park in DC (within Capital Beltway), and Manassas National Battlefield, Virginia but higher than Prince William Forest Park, Virginia (0.05 and 0.03 for 2014 and 2015, respectively). In the western DC suburb of Gaithersburg, MD, Carroll and Cyr (2005) found that each of four adult *I. scapularis* collected from the campus of the National Institute of Standards and Technology (NIST) was infected with *B. burgdorferi* but none of the 16 *I. scapularis* nymphs was infected. The situation at NIST may have been somewhat anomalous, as its wooded habitats had been severely degraded by its dense population of white-tailed deer. *Borrelia miyamotoi* has been reported from *I. scapularis* captured in Baltimore Co., MD (Gatewood Hoen et al. 2009) but was not detected in the present study. Although *Ehrlichia* spp. are known to occur in Maryland *A. americanum* (e.g., *E. chaffeensis* Anderson et al.) (Stromdahl et al. 2000), none of the >300 *A. americanum* that we collected from Greenbelt National Park tested positive. Although our study focused on nymphs, *A. americanum* adults were a significant component of the host-seeking tick population at the park during May and June. Eight adult *A. americanum* we collected were tested for pathogens, but none was infected. Until recent years, *A. americanum* was known mostly as a "nuisance biter." However, removal of well-attached adult *A. americanum* female with its long mouth parts is likely to be a memorable experience.

Waladde and Rice (1982) characterized the host-seeking behavior of *A. americanum* as the hunter type. *Amblyomma americanum* are strongly attracted to CO_2 (Wilson et al. 1972) whereas *I. scapularis* tend to be more sedentary ambushers. Host-seeking *A. americanum* are more readily noticed by the public than are *I. scapularis* (Armstrong et al. 2001). When park visitors and staff notice *A. americanum* at localities where both *A. americanum* and *I. scapularis* occur, an initial encounter with *A. americanum* may induce them to engage in tick-avoidance behaviors and tick checks, which lower the chances of being bitten by *I. scapularis* as well as *A. americanum*.

Four years of sampling populations of host-seeking *I. scapularis* and *A. americanum* nymphs at Greenbelt National Park showed that tick densities differed from year to year; sometimes in only localized ways (e.g., high density foci in new locations) but at other times broadly (e.g., both tick species simultaneously decreasing sharply). A couple of years of low tick densities at a park may give the casual observer a false sense of security. However, as seen at Greenbelt National Park and elsewhere (e.g., Carroll et al. 2009b), tick densities can change, and annual monitoring of tick populations is prudent. Annual sampling for ticks when host-seeking activity for *I. scapularis* and *A. americanum* nymphs starts peaking (late May at Greenbelt National Park), yet before schools close for the summer and the onset of vacation season, gives park managers a window of time to prepare for the impending heightened risks to personnel and visitors associated with a tick irruption. Informational materials could be readied and distributed by park staff and warning signs posted in advance of the seasonal influx of visitors.

Acknowledgments

We thank Mikaila Milton, U.S. Department of Interior (USDI), National Park Service (NPS), National Capital Parks–East for her invaluable cooperation in facilitating our work in Greenbelt National Park, and

Fred Cunningham, Park Manager at Greenbelt National Park during the study for his cooperation. We thank Mark Lehman, USDI, NPS, National Capital Parks–East for providing coordinates and maps of random sampling sites and information on the vegetation communities associated with sampling sites. We are grateful to J. McCrary, A.S. Khan, G. Woodward, A. Abrams, and S. Parsons, USDA, Agricultural Research Center, Beltsville, MD for sampling and collecting ticks and data management.

References Cited

Armstrong, P.M., L.R. Brunet, A. Spielman and S.R. Telford III. 2001. Risk of Lyme disease: Perceptions of residents of a lone star tick-infested community. Bull. World Health Organ. 79: 916–925.

Barnard, D.R., G.A. Mount, H.G. Koch, D.G. Haile and G.I. Garris. 1988. Management of the lone star tick in recreation areas. Agricultural Handbook Number 682. U.S. Department of Agriculture, Springfield, VA.

Bell, C.A. and R. Patel. 2005. A real-time combined polymerase chain reaction assay for the rapid detection and differentiation of *Anaplasma phagocytophilum*, *Ehrlichia chaffeensis*, and *Ehrlichia ewingii*. Diagn. Micr. Infect. Dis. 53: 301–306.

Burgdorfer, W., A.G. Barbour, S.F. Hayes, J.L. Benach, E. Grunwaldt and J.P. Davis. 1982. Lyme disease—A tick-borne spirochetosis? Science 216: 1317–1319.

Carroll, J.F. and T.L. Cyr. 2005. Note on the densities of *Ixodes scapularis* (Acari: Ixodidae) and white-tailed deer on the campus of the National Institute of Standards and Technology, Maryland, USA. Proc. Entomol. Soc. Wash. 107: 973–976.

Carroll, J.F., D.E. Hill, P.C. Allen, K.W. Young, E. Miramontes, M. Kramer, J.M. Pound, J.A. Miller and J.E. George. 2009a. Impact of '4-poster' deer self-treatment devices at three locations in Maryland. Vector-Borne Zoonot. 9: 407–416.

Carroll, J.F., J.M. Pound, J.A. Miller and M. Kramer. 2009b. Sustained control of Gibson Island, MD populations of *Ixodes scapularis* and *Amblyomma americanum* (Acari: Ixodidae) by community-administered '4-poster' deer self-treatment bait station. Vector Borne Zoonotic Dis. 9: 417–421.

Carroll, J.F. and E.T. Schmidtmann. 1996. Silt-fencing as a barrier to dispersal of *Ixodes scapularis* (Acari: Ixodidae) into pastures. J. Med. Entomol. 33: 921–925.

CDC (Centers for Disease Control). 2017. Mortality and morbidity weekly report. Surveillance for Lyme disease – United States, 2008–2015. Surveillance Summaries / November 10, 2017 / 66:1–12.

Childs J.E. and C.D. Paddock. 2003. The ascendancy of *Amblyomma americanum* as a vector of pathogens affecting humans in the United States. Annu. Rev. Entomol. 48:307–337.

Courtney, J.W., L.M. Kostelnik, N.S. Zeidner and R.F. Massung. 2004. Multiple real-time PCR for detection of *Anaplasma phagocytophilum* and *Borrelia burgdorferi*. J. Clin. Microbiol. 42: 3164–3168.

Eisen, L., D. Wong, V. Shelus and R.J. Eisen. 2013. What is the risk for exposure to vector-borne pathogens in United States national parks? J. Med. Entomol. 50: 221–230.

Feldman, K.A., N.P. Connally, A. Hojgaard, E.H. Jones, J.L. White and A.F. Hinckley. 2015. Abundance and infection rates of *Ixodes scapularis* nymphs collected from residential properties in Lyme disease endemic areas of Connecticut, Maryland, and New York. J. Vector Ecol. 40: 198–201.

Gatewood Hoen, A.G., L. Rollend, M. Papero, J.F. Carroll, T.J. Daniels, T.N. Mather, T.L. Schulze, K.C. Stafford and D. Fish. 2009. Effects of control of host-seeking ticks by acaricide self-treatment of white-tailed deer on the prevalence and entomologic risk for *Ixodes scapularis*-borne pathogens. Vector-Borne Zoonot. 9: 431–438.

Ginsberg, H.S. and C.P. Ewing. 1989. Habitat distribution of *Ixodes dammini* (Acari: Ixodidae) and Lyme disease spirochetes on Fire Island, New York. J. Med. Entomol. 26: 183–189.

Goddard, J. and A.S. Varela-Stokes. 2009. Role of the lone star tick, *Amblyomma americanum* (L.), in human and animal diseases. Vet. Parasitol. 160: 1–12.

Johnson, T.L., C.B. Graham, K.A. Boegler, C.C. Cherry, S.E. Maes, M.A. Pilgard, A. Hojgaard, D.E. Buttke and R.J. Eisen. 2016. Prevalence and diversity of tick-borne pathogens in nymphal *Ixodes scapularis* (Acari: Ixodidae) in Eastern National Parks. J. Med. Entomol. 54: 742–751.

Jordan, R.A. and A. Egizi. 2019. The growing importance of lone star ticks in a Lyme disease endemic county: Passive tick surveillance in Monmouth County, NJ, 2006–2016. PLOS ONE 14: e0211778.

Lubelczyk, C.B., S.P. Elias, P.W. Rand, M.S. Holman, E.H. Lacombe and R.P. Smith Jr. 2004. Habitat associations of *Ixodes scapularis* (Acari: Ixodidae) in Maine. Environ. Entomol. 33: 900–906.

Ostfeld, R.S., O.M. Cepeda, K.R. Hazler and M.C. Miller. 1995. Ecology of Lyme disease habitat associations of ticks (*Ixodes scapularis*) in a rural landscape. Ecol. Appl. 5: 353–361.

Paskewitz, S.M., M. Vandermause, E.A. Belongia and J.J. Kazmierczak. 2001. *Ixodes scapularis* (Acari: Ixodidae): Abundance and rate of infection with *Borrelia burgdorferi* in four state parks in Wisconsin. J. Med. Entomol. 38: 33–38.

Pepin, K.M., R.J. Eisen, P.S. Mead, J. Piesman, D. Fish, A.G. Hoen, A.G. Barbour, S. Hamer and M.A. Diuk-Wasser. 2012. Geographic variation in the relationship between human Lyme disease incidence and density of infected host-seeking *Ixodes scapularis* nymphs in the Eastern United States. Am. J. Trop. Med. Hyg. 86: 1062–1071.

Prusinski, M., J. Kokas, K. Hukey, S. Kogut, J. Lee and P. Backenson. 2014. Prevalence of *Borrelia burgdorferi* (Spirochaetales: Spirochaetaceae), *Anaplasma phagocytophilum* (Rickettsiales: Anaplasmataceae), and *Babesia microti* (Piroplasmida: Babesiidae) in *Ixodes scapularis* (Acari: Ixodidae) collected from recreational lands in the Hudson Valley region, New York State. J. Med. Entomol. 51: 226–236.

Pusteria, N., J.B. Huder, C.M. Leutenegger, U. Braun, J.E. Madigan and H. Lutz. 1999. Quantitative real-time PCR for detection of members of the *Ehrlichia phagocytophila* genogroup in host animals and *Ixodes ricinus* ticks. J. Clin. Microbiol. 37: 1329–1331.

Sonenshine, D.E. 1993. Biology of Ticks. Vol. 2. Oxford Univ. Press, New York.

Spielman, A., M.L. Wilson, J.F. Levine and J.F. Piesman. 1985. Ecology of *Ixodes dammini*-borne human babesiosis and Lyme disease. Annu. Rev. Entomol. 30: 439–460.

Straubinger, R.K. 2000. PCR-based quantification of *Borrelia burgdorferi* organisms in canine tissues over a 500-day postinfection period. J. Clin. Microbiol. 2191–2199.

Stromdahl, E.Y. and G.J. Hickling. 2012. Beyond Lyme: Aetiology of tick-borne diseases with emphasis on the south-eastern United States. Zoonoses Public Hlth. 59 (Suppl. 2): 48–64.

Stromdahl, E.Y., M.P. Randolph, J.J. O'Brien and A.G. Gutierrez. 2000. *Ehrlichia chaffeensis* (Rickettsiales: Ehrlichieae) infection in *Amblyomma americanum* (Acari: Ixodidae) at Aberdeen Proving Ground, Maryland. J. Med. Entomol. 37: 344–356.

Van Horn, T.R., S.A. Adalsteinsson, K.M. Westby, E. Biro, J.A. Myers, M.J. Spasojevic, M. Walton and K.A. Medley. 2018. Landscape physiognomy influences abundance of the lone star tick, *Amblyomma americanum* (Ixodida: Ixodidae), in Ozark forests. J. Med. Entomol. 55: 982–988.

Waladde, S.M., M.J. Rice. 1982. The sensory basis of tick feeding behavior, Pp. 71–118. *In* Obenchain, F.D. and Galun, R. (eds.), Physiology of Ticks. Pergamon, New York.

Wilson, J.G., D.R. Kinzer, J.R. Sauer and J.A. Hair. 1972. Chemo-attraction in the lone star (Acarina: Ixodidae). I. Response of different developmental stages to carbon dioxide administered via traps. J. Med. Entomol. 9:245–252.