

Additional U.S. collections of the Gulf Coast tick, *Amblyomma maculatum* (Acari: Ixodidae), from the State of Delaware, the first reported field collections of adult specimens from the State of Maryland, and data regarding this tick from surveillance of migratory songbirds in Maryland

DAVID A. FLORIN¹, R. JORY BRINKERHOFF^{2,3}, HOLLY GAFF^{4,9}, JU JIANG⁵, RICHARD G. ROBBINS⁶, WILLIAM EICKMEYER¹, JAMES BUTLER⁷, DAVID NIELSEN⁷, CHELSEA WRIGHT⁴, ALEXIS WHITE⁴, MAREN E. GIMPEL⁸ & ALLEN L. RICHARDS^{1,5}

¹ Uniformed Services University of the Health Sciences (USUHS), Department of Preventive Medicine and Biometrics, 4301 Jones Bridge Road, Bethesda, MD 20814-4799, U.S.A. E-mail: david.florin@usuhs.edu

² University of Richmond, Department of Biology, Richmond, VA, 23173, U.S.A.

³ University of KwaZulu-Natal, School of Life Sciences, Pietermaritzburg, South Africa

⁴ Old Dominion University, Department of Biological Sciences, Norfolk, VA 23529, U.S.A.

⁵ Naval Medical Research Center, Viral and Rickettsial Diseases Department, 503 Grant Avenue, Silver Spring, MD 20910-7500, U.S.A.

⁶ Armed Forces Pest Management Board, Office of the Deputy Under Secretary of Defense for Installations and Environment, Building 172, U.S. Army Garrison Forest Glen, Silver Spring, MD 20910-1230, U.S.A.

⁷ US Army Public Health Command Region North, 4411 Llewellyn Avenue, Fort George G. Meade, MD, 20755

⁸ Foreman's Branch Bird Observatory, Center for Environment & Society, Washington College, Chestertown, MD 21620, USA

⁹ University of KwaZulu-Natal, School of Mathematics, Statistics and Computer Science, Pietermaritzburg, South Africa

Abstract

This report describes collections of the Gulf Coast tick, *Amblyomma maculatum* Koch (Ixodida: Ixodidae), made during 2013 at the Bombay Hook National Wildlife Refuge (NWR), Delaware, and at two sites in Maryland: the Blackwater NWR and the Chester River Field Research Station (CRFRS). Ticks were collected via field drags, dry ice-baited traps, and/or from the human field researchers (collections of ticks crawling on clothing/boots) at Bombay Hook NWR and Blackwater NWR. A total of 21 *A. maculatum* were successfully collected at Bombay Hook NWR during May 28–30, 2013. Using a genus-specific quantitative real-time polymerase chain reaction (qPCR) assay and species-specific qPCR assays, a single male was found to be positive for the presence of *Rickettsia parkeri* DNA (a spotted fever group rickettsia). The repeated collection of this species in the adult stage at Bombay Hook NWR, the relatively large number collected (n=21), along with its continuity of presence pre- and post-winter, indicate that an established population may now exist at Bombay Hook NWR. A single adult female was collected from a field drag at Blackwater NWR on June 18, 2013; this specimen was negative for the presence of *Rickettsia* spp. DNA (including *R. parkeri* DNA). An adult male was collected on a researcher at CRFRS on August 8, 2013; this specimen was found to be positive for *R. parkeri* DNA. This report also summarizes data from 2008 to 2010 for *A. maculatum* collected during mist netting surveillance of migratory songbirds by the Foreman's Branch Bird Observatory, located at CRFRS: a total of 104 immature *A. maculatum* were collected. The adult specimens of *A. maculatum* collected at Blackwater NWR and at CRFRS are regarded as representing the first documentation of adult field-collected *A. maculatum* within the state. Future sampling is needed at each location to determine if *A. maculatum* is firmly established, the prevalence of *R. parkeri* infection, and the epidemiological risk to humans.

Key words: *Amblyomma maculatum*, *Rickettsia parkeri*, Delaware, Maryland, U.S.A.

Introduction

The Nearctic and Neotropical Gulf Coast tick, *Amblyomma maculatum* Koch, and the associated human pathogen *Rickettsia parkeri* are increasingly being detected in the mid-Atlantic region of the United States (Sumner *et al.* 2007, Teel *et al.* 2010, Fornadel *et al.* 2011, Varela-Stokes *et al.* 2011, Wright *et al.* 2011, Jiang *et al.* 2012). Florin *et al.* (2013) reported the presence of *A. maculatum* and *R. parkeri* at the Bombay Hook National Wildlife Refuge (NWR), DE, after a 2-day collecting trip during May 2012. As a follow-up to that report, subsequent sampling events were conducted at the Bombay Hook NWR, the Blackwater NWR, MD, and the Chester River Field Research Station (CRFRS), Chestertown, MD. In addition, data from the Foreman's Branch Bird Observatory (FBBO) on ticks collected during 2008–2010 bird banding operations at CRFRS are reported here.

Materials and methods

This report describes sampling events at Bombay Hook NWR (May 28–30, 2013), at Blackwater NWR (June 17–19, 2013), and at CRFRS (summer 2013), as well as tick collections from the annual surveillance of songbirds by FBBO at CRFRS (March through November, 2008–2010). Ticks were collected at Bombay Hook NWR and Blackwater NWR by three methods: field drags, dry ice-baited traps, and simply collecting all crawling ticks found on the clothing/boots of the field researchers. This last method was the sole collection technique used at CRFRS during the summer of 2013. All tick specimens were immediately preserved in 70% ethanol and later transported to the Naval Medical Research Center, Silver Spring, MD (collections from Bombay Hook NWR and Blackwater NWR) or to Old Dominion University, VA (collections from CRFRS) to test for the presence of *Rickettsia*. Only ticks morphologically identified as *A. maculatum* were subsequently tested for the presence of *Rickettsia* with a genus-specific quantitative real-time polymerase chain reaction (qPCR) assay and then, if positive, with *Rickettsia parkeri*-specific and *Candidatus Rickettsia andeanae*-specific qPCR assays, as described in Jiang *et al.* (2012).

Migratory and resident songbirds were sampled and checked for ticks during bird banding operations by FBBO (under appropriate state and federal bird collecting permits) at the CRFRS site; data regarding *A. maculatum* are presented here for the period March–November during 2008–2010. Up to 100 Japanese mist nets (6 m and 12 m) were set at this site in ecotone habitats between croplands and upland and wetland woodlots and were open between roughly 0600 hrs and 1300 hrs each day. Time permitting, each captured bird was thoroughly inspected for ticks, and data on bird species, sex, age, wing chord, and body condition were collected. Ticks from each bird were placed in uniquely numbered 2 ml screw-cap vials containing 70% ethanol and were sent to the University of Richmond for species identification (Durdin and Keirans 1996, Keirans and Durdin 1998).

Results and discussion

At Bombay Hook NWR, near Smyrna, DE, successful collection of *A. maculatum* occurred only at the site previously described in Florin *et al.* (2013), although several other sites within Bombay Hook NWR were sampled. The May 28–30, 2013 sampling event produced a total of 21 adult *A. maculatum* (7♂, 14♀), with approximately equal numbers collected by the drag and trap methods. Only a single male specimen was positive for *Rickettsia* genus-specific 17-kDa gene (*htrA*); this specimen subsequently tested positive for the *Rickettsia parkeri*-specific qPCR assay and negative

for the *Candidatus Rickettsia andeanae*-specific qPCR assay. The repeated collection of the adult stage at the same site, the relatively large number collected (n=21), and the continuity of pre- and post-winter presence indicates that an established population may now exist at Bombay Hook NWR. The mist netting operations by the FBBO produced a total of 4350 immature and 39 adult ticks from the surveyed birds, with 130 of these 4389 ticks identified as *A. maculatum* by light microscopy (125 larvae, 5 nymphs). Because of the challenges associated with morphological identification of immature ixodid ticks, especially those that are engorged or damaged when removed from hosts, we used sequence analysis of the 16S gene to confirm species identity on a subset of purported *A. maculatum* larvae and found that 4 of 5 ticks were identified correctly, with one tick confirmed as the congener *A. americanum* (Linnaeus). Thus, we can reasonably estimate that immature *A. maculatum* make up about 2.4% (104/4350) of immature ticks on passerine birds at FBBO, even accounting for errors in morphological species identification due to damaged samples. The birds from which these ticks were recovered represent both resident and migratory species (Table 1), many of which are among the bird species known to be most heavily parasitized by ticks in the eastern United States (Brinkerhoff *et al.* 2011). We failed to detect *A. maculatum* on birds before mid-July, in contrast to other tick species that occur on birds in spring and/or fall months. The temporal pattern of occurrence of immature *A. maculatum* on birds is reflective of the overall picture of tick parasitism, with a peak in immatures occurring in late summer when larval ixodid ticks are most active and abundant (Fig.1). We note that the areas under each of the two curves in Fig.1 are equivalent, and the higher peak of the *A. maculatum* curve represents more restricted seasonal activity rather than a large number of collected ticks of this species.

TABLE 1. Species of songbirds sampled at the Chester River Field Research Station from which immature *A. maculatum* were recovered during 2008 through 2010. Numbers in parentheses indicate numbers of larvae and nymphs, respectively. No adult *A. maculatum* were collected from birds.

Bird species, number of individuals from which ticks were collected	Resident/migratory species¹	Number of immature <i>A. maculatum</i> collected (larvae, nymphs)
Grasshopper Sparrow (<i>Ammodramus savannarum</i>), 3	Migrant	24 (23, 1)
Common Yellowthroat (<i>Geothlypis trichas</i>), 7	Migrant	21 (larvae only)
Song Sparrow (<i>Melospiza melodia</i>), 2	Resident	20 (larvae only)
Wood Thrush (<i>Hylocichla mustelina</i>), 3	Migrant	13 (larvae only)
Carolina Wren (<i>Thyrothorus ludovicianus</i>), 6	Resident	12 (11, 1)
Indigo Bunting (<i>Passerina cyanea</i>), 3	Migrant	11 (larvae only)
Field Sparrow (<i>Spizella pusilla</i>), 5	Resident	9 (larvae only)
Brown Thrasher (<i>Toxostoma rufum</i>), 2	Resident	6 (larvae only)
House Wren (<i>Troglodytes aedon</i>), 3	Migrant	5 (larvae only)
Blue Grosbeak (<i>Passerina caerulea</i>), 1	Migrant	1 (nymph)
Gray Catbird (<i>Dumetella carolinensis</i>), 1	Resident	1 (larva)
Northern Cardinal (<i>Cardinalis cardinalis</i>), 1	Resident	1 (nymph)
Yellow-breasted Chat (<i>Icteria virens</i>), 1	Migrant	1 (nymph)

¹ Resident species are those that are known to overwinter in eastern Maryland, USA; however, individual birds may or may not spend the winter at this field site.

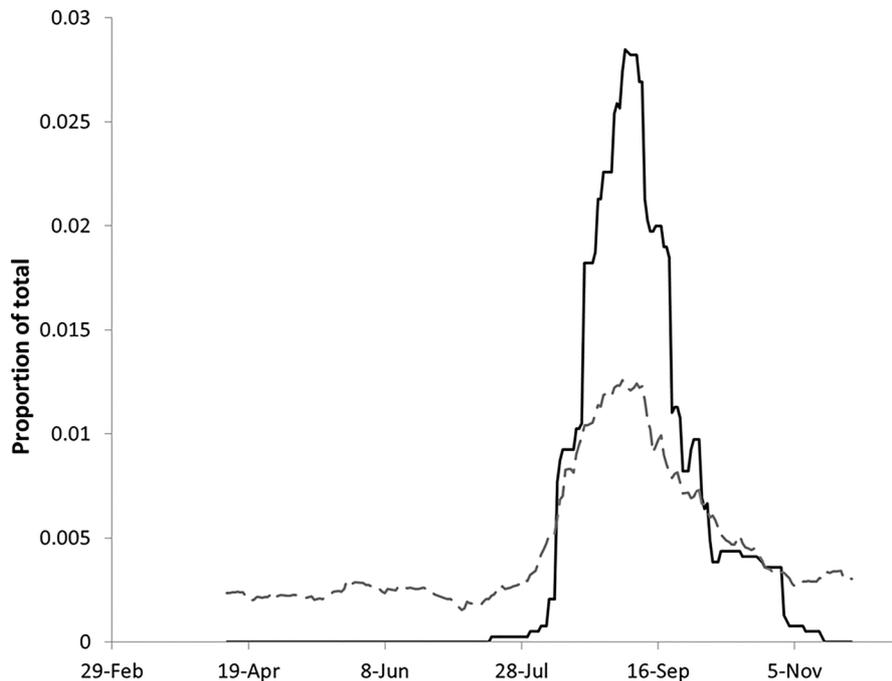


FIGURE 1. Seasonal occurrence of immature *A. maculatum* ($n = 130$ as identified by light microscopy, solid black line) compared to all other tick species collected from songbirds at the Chester River Field Research Station, 2008–2010. Seasonal occurrence of all other ticks ($n = 4259$) in relation to the proportion of birds found with ticks is indicated by the dashed gray line. Curves represent 30-day moving averages of daily proportional tick parasitism on birds, calculated as the proportion of ticks (*A. maculatum* or all other species) collected on a given date.

Although several disparate sites were sampled within Blackwater NWR, only one site resulted in collection of *A. maculatum*: a single adult female was collected via a field drag on June 18, 2013 in highly disturbed, secondary growth habitat (GPS coordinates N38° 26' 40.6", W076° 05' 45.3") located approximately 90 m from the Visitors Center/Gift Shop. This specimen was tested for the presence of *Rickettsia* and found to be negative.

On August 8, 2013, a single adult male *A. maculatum* was found crawling on a human at CRFRS and was subsequently submitted to Old Dominion University for analysis. This tick was found to be positive for *R. parkeri* DNA. The presence of *A. maculatum* in Maryland has been documented by passive surveillance (Stromdahl *et al.* 2011, Jiang *et al.* 2012), yet to our knowledge these specimens from Blackwater NWR and from CRFRS represent the first documented reports of adult *A. maculatum* collected in the field within Maryland and also the first report of an *R. parkeri* infection from a field-collected tick within Maryland (the CRFRS specimen). The occurrence of immature ticks on birds during breeding season is suggestive of an established population of *A. maculatum* at CRFRS even though adults are rarely encountered at this site. Furthermore, the occurrence of this tick on migratory bird species may account for its establishment at this site. Although we did not detect *A. maculatum* on birds during spring migration in our three years of sampling, this tick species has been recovered from migratory birds in the southern and southeastern U.S.A. in spring, fall, and winter (Kinsey *et al.* 2000, Rainwater *et al.* 2007, Robbins *et al.* 2010), and migration could conceivably move enough ticks northward to establish new populations (Scott *et al.* 2001, Ogden *et al.* 2008).

Future sampling is needed at each location to determine whether *A. maculatum* is firmly established, as well as the prevalence of *R. parkeri* infection, the epidemiological risk to humans of Tidewater spotted fever.

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References

- Brinkerhoff, R.J., Folsom-O’Keefe, C.M., Tsao, K. & Diuk-Wasser, M.A. (2011) Do birds affect Lyme disease risk? Range expansion of the vector-borne pathogen, *Borrelia burgdorferi*. *Frontiers in Ecology and the Environment*, 9, 103–110.
<http://dx.doi.org/10.1890/090062>
- Durden, L.A. & Keirans, J.E. (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*. Lanham, Maryland, Entomological Society of America, iv + 95 pp.
- Florin, D.A., Jiang, J., Robbins, R.G. & Richards, A.L. (2013) Infection of the Gulf Coast tick, *Amblyomma maculatum* (Acari: Ixodidae), with *Rickettsia parkeri*: first report from the State of Delaware. *Systematic & Applied Acarology*, 18, 27–29.
<http://dx.doi.org/10.11158/saa.18.1.2>
- Fornadel, C.M., Zhang, X., Smith, J.D., Paddock, C.D., Arias, J.R. & Norris, D.E. (2011) High rates of *Rickettsia parkeri* infection in Gulf Coast ticks (*Amblyomma maculatum*) and identification of “Candidatus *Rickettsia andeanae*” from Fairfax County, Virginia. *Vector-Borne and Zoonotic Diseases*, 11, 1535–1539.
<http://dx.doi.org/10.1089/vbz.2011.0654>
- Jiang, J., Stromdahl, E.Y. & Richards, A.L. (2012) Detection of *Rickettsia parkeri* and Candidatus *Rickettsia andeanae* in *Amblyomma maculatum* Gulf Coast ticks collected from humans in the United States. *Vector-borne and Zoonotic Diseases*, 12, 175–182.
<http://dx.doi.org/10.1089/vbz.2011.0614>
- Keirans, J.E. & Durden, L.A. (1998) Illustrated key to nymphs of the tick genus *Amblyomma* (Acari: Ixodidae) found in the United States. *Journal of Medical Entomology*, 35, 489–495.
- Kinsey, A.A., Durden, L.A. & Oliver, J.H. (2000) Tick infestations on birds in coastal Georgia and Alabama. *Journal of Parasitology*, 86, 251–254.
[http://dx.doi.org/10.1645/0022-3395\(2000\)086\[0251:tiobic\]2.0.co;2](http://dx.doi.org/10.1645/0022-3395(2000)086[0251:tiobic]2.0.co;2)
- Ogden, N.H., Lindsay, L.R., Hanincova, K., Barker, I.K., Birgas-Poulin, M., Charron, D.F., Heagy, A., Francis,

- C.M., O'Callaghan, C.J., Schwartz, I. & Thompson, R.A. (2008) Role of migratory birds in introduction and range expansion of *Ixodes scapularis* ticks and *Borrelia burgdorferi* and *Anaplasma phagocytophilum* in Canada. *Applied and Environmental Microbiology*, 74, 1780–1790.
<http://dx.doi.org/10.1128/aem.01982-07>
- Rainwater, T.R., Robbins, R.G., Ingold, J.L., Platt, S.G. & Walker, T.L. (2007) First record of the Gulf Coast tick, *Amblyomma maculatum* Koch, from Lincoln's Sparrow, *Melospiza lincolni* (Audubon), with a review of tick parasitism of this sparrow. *North American Bird Bander*, 32, 118–119.
- Robbins, R.G., Platt, S.G. & Miller, S.M. (2010) First record of the Gulf Coast tick, *Amblyomma maculatum*, from the lark bunting, *Calamospiza melanocorys*. *North American Bird Bander*, 35, 22–23.
- Scott, J.D., Fernando, K., Banerjee, S.N., Durden, L.A., Byrne, S.K., Banerjee, M., Mann, R.B. & Morshed, M.G. (2001) Birds disperse ixodid (Acari: Ixodidae) and *Borrelia burgdorferi*-infected ticks in Canada. *Journal of Medical Entomology*, 38, 493–500.
<http://dx.doi.org/10.1603/0022-2585-38.4.493>
- Stromdahl, E.Y., Jiang, J., Vince, M. & Richards, A.L. (2011) Infrequency of *Rickettsia rickettsii* in *Dermacentor variabilis* removed from humans, with comments on the role of other human-biting ticks associated with spotted fever group rickettsiae in the United States. *Vector-borne and Zoonotic Diseases*, 11, 969–977.
<http://dx.doi.org/10.1089/vbz.2010.0099>
- Sumner, J.W., Durden, L.A., Goddard, J., Stromdahl, E.Y., Clark, K.L., Reeves, W.K. & Paddock, C.D. (2007) Gulf Coast ticks (*Amblyomma maculatum*) and *Rickettsia parkeri*, United States. *Emerging Infectious Diseases*, 13, 751–753.
<http://dx.doi.org/10.3201/eid1305.061468>
- Teel, P.D., Ketchum, H.R., Mock, D.E., Wright, R.E. & Strey, O.F. (2010) The Gulf Coast tick: a review of the life history, ecology, distribution, and emergence as an arthropod of medical and veterinary importance. *Journal of Medical Entomology*, 47, 707–722.
<http://dx.doi.org/10.1603/me10029>
- Varela-Stokes, A.S., Paddock, C.D., Engber, B. & Toliver, M. (2011) *Rickettsia parkeri* in *Amblyomma maculatum* ticks, North Carolina, USA, 2009–2010. *Emerging Infectious Diseases*, 17, 2350–2353.
<http://dx.doi.org/10.3201/eid1712.110789>
- Wright, C.L., Nadolny, R.M., Jiang, J., Richards, A.L., Sonenshine, D.E., Gaff, H.D. & Hynes, W.L. (2011) *Rickettsia parkeri* in Gulf Coast ticks, southeastern Virginia, USA. *Emerging Infectious Diseases*, 17, 896–898.
<http://dx.doi.org/10.3201/eid1705.101836>

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