TITLE: Avian migrants facilitate invasions of Neotropical ticks and tick-borne pathogens into the United States

RUNNING TITLE: Birds import Neotropical ticks and pathogens to US

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ABSTRACT

Migratory birds have the potential to transport exotic vectors and pathogens of human and animal health importance across vast distances. We systematically examined birds that recently migrated to the United States from the Neotropics for ticks. We screened both ticks and birds for tick-borne pathogens including *Rickettsia* species and *Borrelia burgdorferi*. Over two spring seasons (2013-2014), 3.56% of birds (n = 3,844) representing 42.35% of species examined (n= 85) were infested by ticks. Ground foraging birds with reduced fuel stores were most commonly infested. Eight tick species were identified including seven in the genus *Amblymma* of which only *Ambylomma maculatum/triste* is known to be established in the United States. Most ticks on birds (67%) were Neotropical species with ranges in Central and South America. Additionally, a single *Ixodes* genus tick was detected. A total of 29% of ticks (n= 137) and no avian blood samples (n= 100), were positive for infection with *Rickettsia* species, including *Rickettsia parkeri,* an emerging cause of spotted fever in humans in the southern United States, a species in the group of *Rickettsia monacensis*, as well as uncharacterized species and endosymbionts of unknown pathogenicity. No avian tick or blood samples tested positive for *Borrelia burgdorferi,* the etiologic agent of Lyme disease. Extrapolation of our findings suggests that anywhere from 4 to 39 million exotic Neotropical ticks are transported to the United States annually on migratory songbirds, with uncertain consequences for human and animal health if the current barriers to their establishment and spread are overcome.

INTRODUCTION

The large-scale seasonal movements of migrants provide opportunities for bird-associated parasites to rapidly disperse over large spatial scales, with implications for human and animal health (1, 2). Birds are increasingly recognized for their roles as reservoirs and hosts to vectors for a suite of emerging zoonotic diseases including West Nile virus, Lyme disease, influenza A virus, and H5N1 avian influenza virus (3, 4), and have been implicated in the range expansions or introductions of new pathogens. Additionally, migratory birds have the potential to disperse ectoparasites, and their associated pathogens, over long-distances (5–7).

Migratory birds that over-winter in Central and South America are frequently infested with *Amblyomma* tick species (family Ixodidae), common carriers of *Rickettsia* species parasites (8–11). There are forty-five *Amblyomma* species endemic to the Neotropics (12) where parasitism rates on birds have been found to vary from 6.5% in Panama (10) to 40% in the Brazilian Amazon (9). *Rickettsia* species of bacteria are transmitted to vertebrates by arthropod vectors including *Amblyomma* ticks. The pathogenicity of many tick-borne *Rickettsia* species is unknown but there are over 25 recognized species in the zoonotic spotted fever-group including those that cause Rocky Mountain spotted fever, Mediterranean spotted fever, North Asian tick typhus and Queensland tick typhus (13). Wild birds have been implicated as hosts of *Amblyomma* ticks and *Rickettsia* pathogens in south and central America (8-11), however the significance of wild birds in the epidemiology of these vectors and pathogens remains poorly understood (13).

The hard tick species that attach to birds are characterized by a three host life cycle, in which each active life stage (larva, nymph, adult) will attach to a vertebrate host and feed for a few days to a week after which they drop off from the host, molt to the

next life stage, diapause, and repeat the cycle (14). In the adult stage, the females will mate and feed, drop off the host, and die, whereas males may not require a blood meal. While some tick species are generalists that can readily infest diverse avian or mammalian hosts in any life stage, other tick species have more rigid host preferences. During the off-host time period, where the tick spends a majority of its life, the locomotion of ticks is typically limited to only meters, and accordingly the movement and range expansion of ticks is largely attributed to the movement of the vertebrate hosts during the periods of tick attachment (15). Migratory birds can move hundreds of kilometers including across the Gulf of Mexico and into the United States within a short period of time; for example, the 12 g blackpoll warbler (*Setophaga striata*) can fly up to 2770 km in 3 days (16). If such transcontinental movements coincide with attachment and feeding by ticks, then avian migrations can facilitate the rapid movements of ticks. In the only previous study to systematically examine north-bound spring migrants for ticks immediately upon entry into the United States, Mukherjee et al. (2014) found 3% of songbirds hosted a tick, some of which were infected with spotted fever group *Rickettsia* species. Further, Central and South American *Amblyomma* species have been detected on northward migrating birds as far north as Chicago and Canada (5, 6, 17, 18).

Despite the documented introductions of Neotropical ticks on migratory birds, there is no evidence that these Central and South American tick species are established in the United States, presumably due to biotic or abiotic barriers that prevent their establishment. However, as global climate and other vertebrate host distributions change, the environment in the United States may become more suitable for tropical ticks species, which could change tick-borne disease risk. A recent longitudinal study of European

species found that climate change has influenced the distribution and abundance of parasites associated with many bird species, including ticks (19). In North America, the warming climate has also influenced tick species ranges and phenology (20). For example, *Ixodes scapualris*, a vector of Lyme disease, is thought to be expanding significantly northward in Canada and the threshold numbers of immigrating ticks needed to establish new populations is expected to fall during the coming decades (20, 21).

Understanding the characteristics that lead to bird infestation help predict future invasion scenarios. Ticks typically quest on the low vegetation and either contact potential hosts using an ambush or hunting strategy (14). Accordingly, we expected higher infestation on ground foraging as opposed to canopy foraging birds. Additionally, migrants with reduced fuel stores use more diverse foraging maneuvers, substrates and heights than birds with greater fuel stores (22, 23). Therefore, we expected higher infestation when fuel stores were reduced.

Our objectives were to (i) characterize tick-bird-pathogen associations during spring migration at a high density stopover site on the northern coast of the Gulf of Mexico; (ii) test the hypothesis that tick infestation would be higher for intercontinental migrants that forage closer to the ground and have reduced energetic condition; and (iii) estimate the number of Neotropical ticks entering the United States annually on migratory birds.

METHODS

Bird Capture and Sampling

We investigated the presence of ticks on northward migrants at a high density stopover site on the northern coast of the Gulf of Mexico, The Nature Conservancy's Clive Runnells Family Mad Island Marsh Preserve (Mad Island) in Matagorda County, Texas (Figure 1). Mad Island is intermediate between the breeding and wintering grounds of many species and within the peak spring passage region for eastern songbird migrants in North America. The coastal woodlands at Mad Island provide some of the first resting and refueling habitat for northward migrants after hundreds of miles of non-stop flight across the Gulf of Mexico and many species that occupy different ranges during breeding or wintering occur together there during spring. Therefore, Mad Island is well situated to capture an abundance of many species from broad geographic regions. During spring migration 2013 and 2014, we captured migrants throughout the period of peak passage (24). Birds were captured with mist-nets (12 × 2.6 m or 6 × 2.6 m, 30 mm mesh) placed in wooded habitat. Nets were opened daily between 8:00 and 17:00 CST, except in the case of rain, high winds, or extreme heat. We opened up to 31 individual nets but daily netting effort varied with weather. Upon capture, birds were banded with a unique USGS leg band, weighed to the nearest 0.1g with an electronic scale, and assessed for subcutaneous fat (25).

Birds were scanned for the presence of ticks by systematically searching the ear canals, back of head, mandibular area, perimeter of the eyes, and cloaca (26). A straw was used to blow a stream of air to displace feathers, or feathers were parted with fine-tipped forceps. Ticks were removed with fine-tipped forceps and placed into a dry microcentrifuge tube or in 70% ethanol for later identification. Previously sampled birds were re-examined for ticks when they were re-captured on subsequent days or greater than

three hours later on the same day. All captured birds were searched for ticks except for rare occasions with extreme high capture rates of birds.

During 2014, we collected blood samples from all tick-infested birds and a subset of birds without ticks. We used brachial venipuncture with a 28-gauge needle and capillary tubes or jugular venipuncture with an insulin syringe to collect 50uL of blood (for birds that were 12-19.9 grams) and up to 100uL of blood (for birds ≥20 grams). Blood was expelled into microcentrifuge tubes and stored at -20° C until processing. A variety of bird species that had no detectable tick infestation were sampled opportunistically.

Tick Identification

Ticks were identified to life stage and genus according to morphological keys under a stereomicroscope. Ticks were assigned a relative engorgement score using a scale of 1-4, where a score of 1 indicates a nearly-flat tick removed from a host and a score of 4 indicates a near replete tick. We assumed that tick engorgement and duration of feeding are positively associated based on experimental feeding trials (27), although the absolute duration of attachment was not able to be determined based on the engorgement score. Individual ticks were subjected to DNA extraction using the E.Z.N.A. Tissue DNA Kit (Omega Bio-Tek, Norcross, GA) with the exception a group of >20 larvae that were removed from a single bird which were divided into three pools of 7-9 larvae each prior to extraction. After the addition of lysis buffer, each tick was quartered with a sterile scalpel blade to open the exoskeleton to

118 facilitate lysis. The ticks were incubated at 55° C overnight before the extraction was completed following the manufacturer's
119 instructions.

120 Ticks were identified to species using a PCR-DNA sequencing approach. For all tick samples, PCR to amplify the 12S
121 mitochondrial rDNA was carried out using the T1B and T2A primers resulting in a 360bp product (28). For confirmatory purposes on
122 a subset of samples, an additional PCR to amplify the ITS2 region was carried out using the ITS2-7923-F and ITS2-7923-R primers
123 resulting in a 1.2kb product (29). Reactions were performed in 15ul volumes using 1.5ul of extracted tick DNA as a template with
124 0.5uM of each primer and FailSafe PreMix E buffer and enzyme (Epicentre Technologies Corp., Chicago, IL). PCR products were
125 visualized on 1.5% agarose gels. The positive samples were purified with ExoSAP-IT (Affymetrix, Santa Clara, CA) and Sanger
126 DNA sequencing was performed (Eton Biosciences, San Diego, CA). To facilitate identifications and examine species relationships,
127 sequences were aligned, compared to a national database (NCBI Blast), and neighbor-joining phylogenetic trees were created using
128 Mega 6.0 (30).
129
130 Pathogen Identification
131 We screened ticks and blood samples taken from birds for *Rickettsia* species and *Borrelia burgdorferi*. The full volume of
132 blood from each bird was subjected to DNA extraction as described above, but with a 30 minute lysis step. *Rickettsia* species were
133 detected by amplification of a partial region of the *gltA* gene using the primers RrCS 372 and RrCS 989 resulting in a 617bp product

(31). Confirmatory testing on a subset of samples that tested positive on the initial assay was performed through the amplification of a 632 bp region the *ompA* gene (32). Reactions were run and DNA was sequenced using the same reagents, template volumes, and primer concentrations as indicated above. *B. burgdorferi* was detected using a quantitative, real-time PCR to amplify the 16S rRNA gene using primers and a single Taqman probe specific to *B. burgdorferi* following methods modified from (33). Internal validations in our laboratory yielded a quantitation cycle (Cq) threshold of 33 and below as indicative of positive samples.

Nucleotide sequence accession numbers

The tick and *Rickettsia* sequences obtained from the DNA extracted from tick samples were assigned GenBank accession numbers: KT386301-KT386322.

Characteristics of Infested Birds

We tested expectations that bird infestation with ticks was influenced by life history characteristics including foraging guild, wintering range, and body condition. We categorized species based on where they forage (ground, understory, or sub-canopy and canopy; (34, 35), where they over-winter ("intercontinental migrants" winter south of the Gulf of Mexico in Central and South America and "local birds" winter at the study site on the northern coast of the Gulf of Mexico), and subcutaneous fat stores as an index of body condition (scale from 0 to 5 in which 0 is characterized by no visible fat and 5 is characterized by furcular and

150 abdominal fat deposits that are conspicuously mounded and convex (25). We further categorized intercontinental migratory species by
151 the extent of their stationary non-breeding rages; Central America and the Caribbean (Central), Central America, and South America,
152 and the Caribbean (Central/ South), South America (South). We tested our expectations with zero-inflated negative binomial models
153 for count data (hurdle function in library pscl for R; (36). We used a two part model to account for the high number of zeroes (37).
154 Two part models first model the presence of ticks using a generalized linear model with a logit link and binomial error and then model
155 the abundance of ticks, where they occurred, with a second generalized linear model with a negative binomial distribution and a log
156 link (37). We included foraging guild, wintering range, and body condition as predictive variables.
157
158 Propagule Pressure of Neotropical Ticks entering the United States
159 Propagule pressure is a measure of the frequency and abundance of species introductions, an important determinant of the
160 probability of a non-native species becoming established (38). We estimated the propagule pressure of Neotropical ticks entering the
161 United States on migratory birds using our results for species-specific tick infestation frequency combined with published estimates of
162 bird species abundance for North America (39). We calculated the frequency of infestation for each bird species exclusively by exotic
163 Neotropical tick species (i.e., not including infestations with species known to be established in the United States) for migratory bird
164 species that were frequently examined for ticks (>20 individuals sampled). Additionally, we estimated a range for annual Neotropical
165 tick propagule pressure, using the minimum and maximum values from species-specific infestation frequency across infested bird

166 species. Although our data come from only a single field site, we assume migrants captured at this site are representative of
167 Neotropical migrants entering the United States because (i) migratory birds that stopover along the northern coast of the Gulf of
168 Mexico during spring breed across North American latitudes (40, 41) and (ii) the only other study to systematically examine
169 northbound spring migrants arriving to the United States found a remarkably similar exotic tick infestation prevalence (7).
170
171 RESULTS
172 Tick-Bird Associations
173 During the springs of 2013 and 2014, we screened 3,844 captures of 85 bird species for ticks and found 137 captures (3.56%)
174 of 36 bird species were infested with ticks (Table 1). Tick infestation prevalence did not differ between years (3.82% of 1729 captures
175 from 26 species in 2013; 3.36% of 2115 captures from 25 species in 2014; $z = 0.766$; $P = 0.44$). Infested birds included 26
176 intercontinental migrant species, 9 local wintering species, plus one vagrant species that does not normally occur in Texas (Yellow-
177 green Vireo, *Vireo flavoviridis*). Infested intercontinental migrants over-wintered in Central America and the Caribbean (51
178 individuals of n= 12 species), Central or South America (21 individuals of n= 8 species), and South America (16 individuals of n= 6
179 species). Most infested birds carried one detected tick (n=114), 22 birds carried two to five individual ticks, and one bird, an Acadian
180 flycatcher (*Empidonax virescens*), carried 27 ticks (Figure 2).

We collected and identified 178 individual ticks. Ticks were exclusively larvae and nymphs. Based on mitochondrial 12S rDNA and ITS-2 sequences, we identified seven different *Amblyomma* species and a single *Ixodes* species (Table 1). Due to similar appearance and identical DNA sequences at the loci we examined, we could not differentiate between *A. maculatum* and *A. triste*, as was the case in a recent study of bird ticks (7). The single *Ixodes* tick shared 96% sequence homology with *I. minor* removed from a bird in Costa Rica (KF702338) based on analysis of the 12S rRNA, and shared >90% sequence homology with *I. dentatus* removed from a bird in Chicago (JQ868583) based on analysis of the ITS-2 region. Ticks collected off four birds were determined to be in the genus *Amblyomma* but could not be identified to species. On the basis of 12S rRNA sequence analysis, these four sequences were identical to each other and shared 90% sequence homology with *A. calcaratum* removed from a southern tamandua in Peru (*Tamandua tetradactyla*) (AY225322) and 90% sequence homology with *A. dubitatum* removed from *Didelphis albiventris* in Brazil (AY342258). On the basis of ITS-2 sequence analysis performed on two of the unknown *Amblyomma* ticks, the ticks were identical to each other and shared >93% sequence homology with *A. aureolatum* in Brazil (AF469611). No sequence was obtained from ticks collected off three birds. When multiple ticks were collected from the same bird and processed separately they were always identified to be the same tick species (n= 19 birds). Of the tick species for which we collected more than three individuals, mean engorgement scores were lowest for *A. maculatum/ triste* (2.02 ± 0.70 SD) yet generally similar across all species (*A. ovale*, 2.13 ± 0.64; *A. longirostre*, 2.64 ± 0.96; *A. nodosum*, 2.65 ± 0.94).

196 Migrants from Central America and the Caribbean were infested with all seven *Amblyomma* species while migrants from
197 South America were infested with only three of those species (*A. longirostre*, *A. nodosum, A. geayi*). The single *Ixodes* spp. tick was
198 detected on a migrant from South America (Grey-cheeked Thrush, *Catharus minimus*). *A. auricularium* and *A. coelebs* were only
199 detected on migrants from Central America. Some tick species infested more than 10 bird species (*A. longirostre*, *A. maculatum/*
200 *triste*, *A. nodosum*) while others infested two to three bird species (*A. auricularium*, *A. coelebs*, and *A. geayi*; Table 1).
201 Of the 1,241 times birds were recaptured and rechecked for ticks, a single tick was removed from each of 10 different
202 recaptured birds, one to 10 days after their initial capture. Of these, seven *A. maculatum/ triste* nymphs with engorgement scores of 1
203 to 3 were detected on avian species with winter ranges that include our study site. Two were *A. longirostre* larvae with engorgement
204 scores of 2 and 3 and were detected on two intercontinental migrants (Red-eyed Vireo, *Vireo olivaceus* and Tennessee Warbler,
205 *Oreothlypis peregrine*) one and two days after the initial capture. Finally, one *A. nodosum* nymph with engorgement score of 2 was
206 detected on an intercontinental migrant (Painted Bunting, *Passerina ciris*) recaptured one day after the initial capture. Eight birds
207 were initially banded and checked for ticks in 2013 and recaptured and checked for ticks in 2014; all were local wintering birds
208 checked for ticks on a combined total of 24 occasions and no ticks were found at any time.
209 Two ticks were collected off bird banders on May 10, 2013. They were both identified as adult *Dermacentor variabilis,* one
210 female and one male.
211

212 Bird and Tick Infection

213 We screened 137 ticks for *Rickettsia* species and *Borrelia burgdorferi*. Thirty eight individual ticks of six *Amblyomma spp.*
214 were infected with at least five different *Rickettsia* species (Table 2, Figure 3). *Rickettsia parkeri/ rickettsii* infected at least two
215 Neotropical tick species (*A. nodosum* and *A. ovale*) collected off of four migratory bird species; sequencing of *gltA* and *ompA* did not
216 distinguish between these two pathogenic species. *Rickettsia amblyommii* infected three Neotropical tick species (*A. auricularium, A.*
217 *geayi, A. longirostre*) on five intercontinental migrant bird species. The *Rickettsia* endosymbiont of *A. maculatum* infected exclusively
218 the *A. maculatum/ triste* ticks collected off of two local and four intercontinental migratory bird species. *Rickettsia* spp. - Brazil
219 infected two Neotropical tick species *Amblyomma geayi* and *Amblyomma longirostre* collected off of nine migratory bird species. The
220 single *Ixodes* spp. larva we collected off of the migratory Gray-cheeked Thrush (*Catharus minimus*) was infected with a *Rickettsia*
221 spp. that is in the group of *Rickettsia monacensis*, a spotted fever group human pathogen in Europe and Asia, on the basis of analysis
222 of the *gltA* and *ompA* genes. The detected species shared >99% sequence homology to *R. monacensis* from human blood in South
223 Korea (KC993860) and a *Rickettsia* spp. from a questing *I. ricinus* from Slovakia (AF140706) based on *gltA* analysis, and >97%
224 sequence homology to various *Rickettsia monacensis*, endosymbionts, and undescribed strains from *Ixodes* species in the southern
225 United States, central and South America (KJ507217, EF689735, KF702334, GQ902957, KF831361, EU544297, AF031535, and
226 HM161773) on the basis of *ompA* analysis. No ticks tested positive for infection with *Borrelia burgdorferi*.

227 A total of 238 blood samples from 38 species were collected from birds in 2014, representing 11% of all birds that were
228 checked for ticks in that year (n= 2115; Table 1). Fifty of the samples (21%) were from infested birds (70% of infested birds in 2014)
229 and the remaining 188 were from un-infested birds. None of the 238 samples were positive for *Rickettsia* spp. using the *gltA* assay.
230 We subjected a random subset of 100 DNA extracts from these blood samples to the secondary *ompA* assay, and none were positive.
231 No blood samples tested positive for infection with *Borrelia burgdorferi*.
232
233 Characteristics of Infested Birds
234 We tested our expectations regarding life history characteristics of infested birds, with samples from 56 intercontinental
235 migrant (n=3,177 captures) and 28 local species (n= 665). We sampled 21 ground foraging (n= 912), eight understory (n= 1148), and
236 21 canopy/ subcanopy species (n= 1784). Local and intercontinental migrant groups included species in each of the three foraging
237 groups. We sampled birds with completely depleted fuel stores (fat score = 0, n= 1634), low fuel stores (fat score = 1, n= 1259) and
238 moderate to considerable fuel (fat score = 2 - 4, n= 949). Fat score was not recorded for two individuals and the Acadian Flycatcher
239 with 27 ticks was removed from analyses as an outlier. Migratory status, foraging guild and energetic condition did not influence the
240 abundance of ticks for birds that were infested (Table 3). However, birds with reduced fuel stores that foraged closer to the ground
241 were most likely to be infested (Table 3). Intercontinental migrants were not more likely to be infested than local wintering birds
242 (Table 3).

243

244 Propagule Pressure of Neotropical Ticks entering the United States

245 Twenty-five migrant species screened were infested with one or more exotic Neotropical tick species (*Amblyomma*
246 *auricularium, A. coelebs, A. geayi, A. longirostre, A. nodosum,* and *A. ovale*). Nineteen of those species were screened sufficiently to
247 derive frequencies of infestation with Neotropical ticks (128.32 ± 153.40 SD birds sampled) and species-specific frequency of
248 infestation varied (0.036 ± 0.02 SD ticks). Propagule pressure of Neotropical ticks entering the United States annually on migratory
249 songbirds was over 19 million (19,418,653), derived from species-specific infestation frequencies and North American abundance
250 estimates. Minimum (0.008; Gray Catbird, *Dumetella carolinensis*) and maximum (0.074; Summer Tanager, *Piranga rubra*)
251 Neotropical tick infestation frequencies applied across infested species resulted in low and high estimates of over 4 million
252 (4,224,240) and over 39 million (39,074,220) propagule of Neotropical ticks entering the United States annually on migratory birds.

253

254 DISCUSSION

255 Every spring, birds migrate northward into the United States from Central and South America, where they spent the winter.
256 After crossing the Gulf of Mexico, migrants congregate in coastal habitats before moving on to breeding areas throughout North
257 America (40, 41). Migrating songbirds can move thousands of kilometers in just a few days (16, 42), and we found 3% of migrants in
258 coastal Texas harbored ticks across the Gulf of Mexico, over two-thirds (67%) of which were Neotropical tick species not known to

occur in the United States. Extrapolation of our data yields an estimate of a bird-associated propagule pressure of over 19 million exotic Neotropical ticks imported to the United States each spring. Although this extrapolation is limited by uncertainties in estimates of North American breeding bird abundance (39), our estimate of exotic tick propagule pressure is likely conservative given that our calculation only includes data from 19 infested birds species that were commonly captured, combined with a previous study's finding that 33% of ticks on birds may fail to be detected by bird banders (4).

There have been a growing number of observations of Neotropical ticks on birds throughout the eastern half of the United States and Canada (4–6, 17, 18, 43, 44). The infestation prevalence we found is remarkably similar to that found in the only other standardized survey of spring migrants arriving to the United States; that found 2.4% of migrants were infested (7). Nonetheless, no datasets are available to provide evidence that any of these Neotropical tick species have established locally within the United States. We found less than 1% of locally recaptured birds were infested, and when they were it was primarily with *Amblyomma maculatum/ triste;* both *A. maculatum* and *A. triste* are known to be established in Texas (45). Although we detected two Neotropical ticks (*A. longirostre and A. nodstrom*) on recaptured birds, our methods do not allow determination of whether these ticks failed to be detected on first capture or whether they could have been acquired locally. Despite the activity of juvenile *A. americanum* across the southern states in the spring, we did not detect this species on birds in our study. At least three reasons may contribute to its lack of detection in our study: (i) our work was in coastal marsh habitat and not the preferred woodland habitat of *A. americanum* (46); (ii) passerine birds

seem to be less utilized by *A. americium* relative to other vertebrate species (47); and (iii) the majority of birds we examined had recently arrived from the southern tropics where *A. americanum* is not distributed (46).

The pathway of species invasion includes five stages, in which (i) an exotic species is in the invasion pathway; (ii) it is transported and released alive; (iii) a new population establishes; (iv) the population spreads; and (v) ecological, human health, or economic impacts result (48). Our data support that the first two stages of the invasion pathway are underway. Barriers to establishment may be both biotic and abiotic, including host species or climactic limitations. For example, although these Neotropical ticks feed on diverse wild bird species in their larval and nymphal stages, the adult life stages typically feed upon wild mammalian hosts that do not exist in the southern United States. For example, *A. coelebs* feeds on tapirs (49); *A. longirostre* feeds on porcupines (50); and *A. nodosum* feeds on Neotropical anteaters (51). In contrast, the host range of *A. ovale* in the Neotropics includes felids rodents and carnivores (12, 52, 53) and *A. auricularium* feeds on armadillos (Dasypodidae) (52, 54), all of which are abundant in south Texas. Nonetheless, an adult *A. longirostre* was recently found crawling on a propane tank outside of a home in Oklahoma in the fall, which could represent a bird-imported nymph that arrived in the spring and successfully molted (55).

Ongoing changes to the climate may alter the species ranges and phenology of tropical tick species, resulting in unknown disease consequences. A longitudinal study found that climate change has already influenced the abundance and distribution of tick species associated with European birds (19) and models found *Ixodes scapualris* will expand significantly northward Canada (20, 21). Further, climate warming trends correlated with as much as three weeks earlier activity of *I. scapularis* in the spring (20). At the same

time, migratory songbirds have not advanced their arrival timing across the Gulf of Mexico over the past two decades but they are arriving earlier to breeding grounds throughout North America (24). Therefore, migrants may be compensating for advancing spring phenology by migrating faster within North America (56), potentially moving further during the period of time when they are infested with Neotropical ticks. Such distributional and seasonal expansions place ticks in contact with additional humans and afford more opportunities for pathogen transfer.

An increasing number of spotted fever group *Rickettsia* species are recognized to cause disease in humans (13, 57), including *R. parkeri* which we detected in ticks on northward migrants. *R. parkeri* was first described in 1939 in *A. maculatum* ticks from Texas, and has only recently been implicated in human disease in the southern United States (58) where cross-reaction with *R. rickettsia* (agent of Rocky Mountain Spotted Fever) occurs and the human burden of disease is therefore difficult to discern. The role of birds in the ecology of *R. parkeri* is unknown, but *R. parkeri*-like organisms have been detected from at least three species of bird-derived Neotropical *Amblyomma* ticks in Mexico and Brazil (59, 60). Further, the possible pathogenic effects for humans of Neotropical *Rickettsia* species are largely unknown (61). Human-biting has been documented by many of the Neotropical ticks we detected (e.g., 62, 63), suggesting that the establishment of these ticks could be associated with human biting and the opportunity for bridging of pathogens with human health consequences.

The single *Ixodes* spp. larva in our study was infected with a *Rickettsia* in the group of *R. monacensis,* a spotted fever group pathogen that is associated with *Ixodes ricinus* ticks in Europe and North Africa (64, 65) and is the causative agent of a Mediterranean

spotted fever-like illness in humans (66). Our finding may reflect a rickettsemic bloodmeal within the tick, indicative of an infected and potentially reservoir-competent avian host, or it may reflect a systemically infected tick that could have been infected transovarially from an infected female tick. The infected tick was removed from a Gray-cheeked thrush, a species that winters in South America east of the Andes and breeds in spruce forests in Alaska and across northern Canada. To our knowledge, this agent has not previously been reported in the Americas (57).

No avian tick or blood sample was infected with *B. burgdorferi* in our study. Although each Neotropical *Amblyomma* species in our study has not been evaluated as a candidate vector of *B. burgdorferi*, the overwhelming evidence from decades of work with *A. americanum* indicates that *A. americanum* is not a vector of *B. burgforgeri* due to a borreliacidal agent in the saliva (67), and it is extended that the genus *Amblyomma* is not likely contributing to Lyme disease epidemiology or ecology. Testing for *B. burgdorferi* in our study was not performed to identify vectors, but rather to potentially learn about enzootic maintenance of the pathogen in local or migratory birds and their ticks given that spirochetes in the avian blood could have been detected by our approach of testing the blood directly or testing the engorged ticks. Birds play a key role in the ecology of Lyme disease through contributing to the range expansion of ticks and maintaining *B. burgdorferi* in the environment in areas where Lyme disease is recognized as endemic or emerging (4, 26, 68–70). Regional studies of pathogen prevalence are increasingly important from an ecological and human health perspective, especially considering that the annual movement patterns of many of the birds connect Lyme-endemic and nonendemic geographic zones.

322 We found that ground-foraging birds were more likely to be infested with ticks than those that forage elsewhere - a finding that
323 is congruent with many other studies over different geographic regions (e.g., 7, 70, 71) and reflects the ground-level host seeking
324 behavior of ticks (14). We also found that birds with reduced fat stores were more likely to harbor ticks. When fuel stores are depleted
325 during migration, birds expand their foraging heights and substrates (22, 23) and may therefore be increasingly exposed to questing
326 ticks on low vegetation. Although it is possible for a migrant to carry a tick from South America in a few days, migrants from South
327 America may also acquire ticks during migratory stopovers in Central America. Three of the tick species we detected occur broadly
328 across Central and South America, *A. longirostre, A. nodosum,* and *A. maculatum/ triste* (51) and these species infested many bird
329 species from both Central and South America as well as local non-migratory birds. Six of the seven *Amblyomma* species were detected
330 on bird species that winter in Central America and three *Amblyomma* species that occur in both Central and South America, A.
331 *auricularum. A. coelebs,* and *A. ovale*, were only detected on migrants from Central America. Only *A. geayi* was detected exclusively
332 on canopy foraging migrants from South America (Red-eyed Vireo and Scarlet Tanager). It is possible that these long-distance
333 migrants transported *A. geayi* from South America, where they are known to infest birds in the Amazon (9), to the United States but *A.*
334 *geayi* is also known to occur in Central America (72). The broad geographic distribution of the tick and bird species in this study
335 limits our ability to identify the geographic location of infestation.

336 Exotic Neotropical ticks and associated pathogens are being transported and presumably released alive in the United States.
337 Recommendations specific to this stage in the invasion pathway include monitoring for early invasions to allow a rapid response, and

providing authority and funding for eradication and control programs (48). However these introduction events via the natural migrations of native bird species have likely been ongoing for millenia with no perceived economic or health imapact to date given the apparent lack of establishment of the invading species. Nonetheless, as climates and ranges of potential vertebrate host species change, future studies to elucidate the origin and destination of ticks and pathogens carried by birds, as well as detailed studies of local vertebrate hosts that could be used to support establishing populations of exotic ticks and pathogens, will be important for more fully understanding avian migration in the field of disease ecology.

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353 authors provided comments. E.B.C and P.P.M designed and coordinated field work and S.A.H and L.D.A designed and performed lab
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511 TABLES

512 Table 1. Bird species captured on the northern coast of the Gulf of Mexico during spring migration screened for ticks (2013- 2014).
513 Species with at least one infested individual are shown (3,844 individuals of 85 species screened). Bird species were categorized
514 according to foraging height during migration (Ground, Understory, and Canopy/ Subcanopy) and whether their winter range was
515 south of the Gulf of Mexico or included the study region (Intercontinental Migrant or Local, respectively). We further categorized
516 migrants on the extent of their wintering range (Central America and the Caribbean, Central and South America and the Caribbean, or
517 South America). We captured one infested vagrant species from Central America that does not normally occur in Texas.

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					Tick Species										
Bird Species	**Family** [1]	**Foraging** [2]	**Range** [3]	**Sampled for ticks** [4]	***Amblyomma auricularium***	***Amblyomma coelebs***	***Amblyomma geayi***	***Amblyomma longirostre***	***Amblyomma maculatum/ triste***	***Amblyomma nodosum***	***Amblyomma ovale***	***Amblyomma* spp.**	***Ixodes* spp.**	**Unknown tick species**	**Total infested (%)**
Eastern Wood Pewee (*Contopus virens*)	Tyrannidae	C	M, S	49				1		1					2 (4.1)
Acadian Flycatcher (*Empidonax virescens*)	Tyrannidae	C	M, C/S	16				1							1 (6.3)
Yellow-green Vireo (*Vireo flavoviridis*)	Vireonidae	C	M, V	1				1							1 (100)
White-eyed Vireo (*Vireo griseus*)	Vireonidae	C	L	134				1						1	2 (1.5)

Philadelphia Vireo (*Vireo philadelphicus*)	Vireonidae	C	M, C	32				1							1 (3.1)
Red-eyed Vireo (*Vireo olivaceus*)	Vireonidae	C	M, S	148			1	6				1			8 (5.4)
House Wren (*Troglodytes aedon*)	Troglodytidae	U	L	14					1						1 (7.1)
Veery (*Catharus fuscescens*)	Turdidae	G	M, S	42						1		1			2 (4.8)
Grey-cheeked Thrush (*Catharus minimus*)	Turdidae	G	M, S	39				1				1	1		3 (7.7)
Swainson's Thrush (*Catharus ustulatus*)	Turdidae	G	M, C/S	158		1	1	7		1					10 (6.3)
Wood Thrush (*Hylocichla mustelina*)	Turdidae	G	M, C	47					1	1	2			1	5 (10.6)
Gray Catbird	Mimidae	U	M, C	646	1	1		1	1		2				6

(*Dumetella carolinensis*)															(0.9)
Brown Thrasher (*Toxostoma rufum*)	Mimidae	G	L	7					1						1 (14.3)
Ovenbird (*Seiurus aurocapilla*)	Parulidae	G	M, C	42	1										1 (2.4)
Worm-eating Warbler (*Helmitheros vermivorum*)	Parulidae	U	M, C	33				2							2 (6.1)
Northern Waterthrush (*Parkesia noveboracensis*)	Parulidae	G	M, C/S	78					1						1 (1.3)
Black-and-white Warbler (*Mniotilta varia*)	Parulidae	C	M, C/S	89				3		1					4 (4.5)
Prothonotary Warbler (*Protonotaria citrea*)	Parulidae	C	M, C/S	14				1							1 (7.1)
Tennessee Warbler (*Oreothlypis peregrina*)	Parulidae	C	M, C/S	208				1		1					2 (1.0)

Kentucky Warbler (*Geothlypis formosa*)	Parulidae	U	M, C/S	59					4	1	1				6 (10.2)
Common Yellowthroat (*Geothlypis trichas*)	Parulidae	U	L	260						1					1 (0.4)
Hooded Warbler (*Setophaga citrina*)	Parulidae	U	M, C	90				1	1	1	1				4 (4.4)
Bay-breasted Warbler (*Setophaga castanea*)	Parulidae	C	M, S	13				1							1 (7.7)
Chestnut-sided Warbler (*Setophaga pensylvanica*)	Parulidae	C	M, C/S	19				2							2 (10.5)
Yellow-breasted Chat (*Icteria virens*)	Parulidae	U	M, C	1							1				1 (100)
White-throated Sparrow (*Zonotrichia albicollis*)	Emberizidae	G	L	1					1						1 (100)
Savannah Sparrow (*Passerculus*	Emberizidae	G	L	7					1						1

sandwichensis)															(14.3)
Swamp Sparrow (*Melospiza georgiana*)	Emberizidae	G	L	12					3						3 (25.0)
Lincoln's Sparrow (*Melospiza lincolnii*)	Emberizidae	G	L	58					2			1			3 (5.2)
Rose-breasted Grosbeak (*Pheucticus ludovicianus*)	Cardinalidae	C	M, C	102						1					1 (1.0)
Blue Grosbeak (*Passerina caerulea*)	Cardinalidae	C	M, C	19						1					1 (5.3)
Indigo Bunting (*Passerina cyanea*)	Cardinalidae	G	M, C	397				1	7	7				1	16 (4.0)
Scarlet Tanager (*Piranga olivacea*)	Cardinalidae	C	M, S	46			1	1							2 (4.3)
Painted Bunting (*Passerina ciris*)	Cardinalidae	C	M, C	157	1					2					3 (1.9)

Summer Tanager (*Piranga rubra*)	Cardinalidae	C	M, C/S	54				2		2					4 (7.4)
Northern Cardinal (*Cardinalis cardinalis*)	Cardinalidae	C	L	54					8						8 (14.8)
Total				**3097**	**3**	**2**	**3**	**35**	**32**	**22**	**7**	**4**	**1**	**3**	**112 (2.9)**

[1] G= Ground, U= Understory, C= Canopy/ Subcanopy

[2] Stationary non-breeding range L= Local or M= Migrant from C= Central America and the Caribbean, C/S=Central and South America and the Caribbean, S= South America, V= Vagrant from Central America[3] An additional 698 individuals of 49 species were sampled for ticks and were not infested.

533 Table 2. Ticks collected off of birds on the northern coast of the Gulf of Mexico during the spring of 2013 and 2014 and sampled for
534 infection with *Rickettsia* species and *Borrelia burgdorferi*. Number (%) of tick species infected with five *Rickettsia* species. No ticks
535 tested positive for *Borrelia burgdorferi*.

		Number infected (%)				
Tick Species	**Number of ticks tested**	***Rickettsia amblyommii***	**Rickettsia endosymbiont of *A. maculatum***	***Rickettsia monacensis***	***Rickettsia parkeri/ rickettsii***	***Rickettsia* spp. Brazil**
Amblyomma auricularium	7	2 (28.6)				
Amblyomma coelebs	2					
Amblyomma geayi	3	1 (33.3)				2 (66.7)
Amblyomma longirostre	42	5 (11.9)				16 (38.1)
Amblyomma maculatum/ triste	43		8 (18.6)			
Amblyomma nodosum	26				1 (3.8)	
Amblyomma ovale	8				2 (25)	

Amblyomma spp.	5				1 (20)	
Ixodes spp.	1			1 (100)		
Total	**137**	**8 (5.8)**	**8 (5.8)**	**1 (0.7)**	**4 (2.9)**	**18 (13.1)**

536

537

538 Table 3. Factors influencing the occurrence and abundance of tick infestation on birds
539 sampled during spring migration. The binomial model tests for relationships in the
540 presence or absence of ticks and the count model tests for relationships within positive
541 samples (n= 3839 samples, *df*= 11).
542

	Binomial model			Count model		
Effect	Estimate	X^2	*P*	Estimate	X^2	*P*
Intercept	-2.62 ± 0.29	--	--	-9.24 ± 99.60	--	--
Intercontinental migrants[1]	-0.16 ± 0.26	0.37	0.546	-1.01 ± 0.65	0.52	0.104
Understory[2]	-0.88 ± 0.27	14.20	0.001	0.40 ± 0.62	1.44	0.486
Canopy and Subcanopy[2]	-0.71 ± 0.22	14.20	0.001	-0.41 ± 0.60	1.44	0.486
Fat[3]	-0.37 ± 0.13	7.89	0.005	-0.20 ± 0.34	0.37	0.543

543 [1] as compared to birds that over-wintered locally
544 [2] as compared to ground foraging birds
545 [3] Fat score increased from completely depleted (score 0) to low (score 1) to moderate and
546 considerable fuel (scores 2-4)
547

548 FIGURE HEADINGS

549 Figure 1. Birds were captured during northward spring migration on the northern coast of the Gulf of Mexico at The Nature
550 Conservancy's Clive Runnells Mad Island Marsh Preserve in Matagorda County, Texas (circle). (Base map copyright ESRI.)

551

552 Figure 2. Acadian Flycatcher (*Empidonax virescens*) captured on 23 April 2014 carrying 27 larval ticks around its eyes. The identity
553 of pooled ticks was molecularly confirmed as *Amblyomma longirostre* infected with *Rickettsia amblyommii*. Photo by permission from
554 Tim Guida.

555

556 Figure 3. Phylogenetic relationships of the *Rickettsia* species detected in ticks removed from birds in Texas, 2013-2014, based on
557 partial *gltA* gene sequences (492 positions) and inferred by the Neighbor-joining method. The percentage of replicate trees in which
558 the associated taxa clustered together in the bootstrap test (500 replicates) are shown next to the branches when 60% or greater. The
559 evolutionary distances were computed using the maximum composite likelihood method and are in the units of the number of base
560 substitutions per site. Sequences generated in the current study are in bold.