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Original article

Importation of exotic ticks and tick-borne spotted fever group rickettsiae into the United States by migrating songbirds

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ABSTRACT

Birds are capable of carrying ticks and, consequently, tick-transmitted microorganisms over long distances and across geographical barriers such as oceans and deserts. Ticks are hosts for several species of spotted fever group rickettsiae (SFGR), which can be transmitted to vertebrates during blood meals. In this study, the prevalence of this group of rickettsiae was examined in ticks infesting migratory songbirds by using polymerase chain reaction (PCR). During the 2009 and 2010 spring migration season, 2064 northward-migrating passerine songbirds were examined for ticks at Johnson Bayou, Louisiana. A total of 91 ticks was removed from 35 individual songbirds for tick species identification and spotted fever group rickettsia detection. Ticks were identified as *Haemaphysalis juxtakochi* ($n = 38$, 42%), *Amblyomma longirostre* ($n = 22$, 24%), *Amblyomma nodosum* ($n = 17$, 19%), *Amblyomma calcaratum* ($n = 11$, 12%), *Amblyomma maculatum* ($n = 2$, 2%), and *Haemaphysalis leporispalustris* ($n = 1$, 1%) by comparing their 12S rDNA gene sequence to homologous sequences in GenBank. Most of the identified ticks were exotic species originating outside of the United States. The phylogenetic analysis of the 71 *ompA* gene sequences of the rickettsial strains detected in the ticks revealed the occurrence of 6 distinct rickettsial genotypes. Two genotypes (corresponding to a total of 28 samples) were included in the *Candidatus* Rickettsia amblyommii clade (less than 1% divergence), 2 of them (corresponding to a total of 14 samples) clustered with *Rickettsia* sp. "Argentina" with less than 0.2% sequence divergence, and 2 of them (corresponding to a total of 27 samples), although closely related to the *R. parkeri–R. africae* lineage (2.50–3.41% divergence), exhibited sufficient genetic divergence from its members to possibly constitute a new rickettsial genotype. Overall, there does not seem to be a specific relationship between exotic tick species, the rickettsiae they harbor, or the reservoir competence of the corresponding bird species.

Introduction

Ticks have a diverse range of vertebrate hosts and can acquire and transmit microorganisms while blood feeding. Ticks can transmit pathogens to humans and animals including bacteria, viruses, and protozoans (Sonenshine, 1993). Disease agents can remain in the tick from one life stage to the next (transstadial transmission). Additionally, infected female ticks can also pass pathogenic microbes to the next generation via their eggs (transovarial or vertical transmission) (Burgdorfer and Brinton, 1975).

Rickettsia spp. are obligate intracellular organisms, which are found worldwide and can infect arthropod vectors. They have traditionally been genetically and antigenetically classified into the spotted fever group (SFG) and the typhus group (TG). Several strains of the SFG rickettsiae have been associated worldwide with human disease and are transmitted by ticks (Roux and Raoult, 2000; Labruna et al., 2004).

Migratory birds are known to serve as long-distance carriers for several ectoparasites and microbial agents (Hoogstraal et al., 1961, 1963; Anderson et al., 1986; Olsén et al., 1995; Olsen et al., 1998; Palmgren et al., 1997; Björsdorff et al., 2001; Scott et al., 2001; Morshed et al., 2005; Santos-Silva et al., 2006; Elfving et al., 2010; Movila et al., 2010; Hildebrandt et al., 2010; Hasle et al., 2011). Birds often serve as hosts of immature ticks and represent a reservoir for human and other animal tick-borne pathogens (Hubalek, 2004).

Millions of songbirds travel long distances between temperate breeding areas in the continental United States and tropical wintering areas in the Caribbean, in Mexico and in Central and South America (Rappole et al., 1995). Migrating songbirds provide a means for ectoparasites, like ticks, to be dispersed across thousands

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of miles. Several species of ticks of the genus *Argas*, *Ornithodoros*, *Ixodes*, *Amblyomma*, and *Haemaphysalis* commonly parasitize birds (Barros-Battesti et al., 2006; Ogrzewalska et al., 2008; Movila et al., 2010; Hasle et al., 2011). However, the literature concerning spotted fever group rickettsial infections of ticks found on northward migrating songbirds in the United States is scant, and the potential role of songbirds as reservoirs or amplifying hosts for exotic spotted fever group rickettsiae has not been studied.

The main objective of this study was to investigate the prevalence of different SFG rickettsial species associated with ticks infesting northward-bound Neotropical–Nearctic-migratory songbirds collected at a Mississippi flyover (LA) using molecular approaches.

Materials and methods

Study site

This study was conducted during the spring 2009 and 2010 migration seasons (15 March–15 May) at a long-term migratory bird study site near Johnson Bayou, Louisiana (29°45′ N, 93°37′ W). Johnson Bayou is comprised of woodlands, known as chenier (French for "oak forest"), along the northern coast of the Gulf of Mexico and migratory birds often stop here to rest and feed following their flight across the Gulf of Mexico (Moore, 1999). The dominant tree species are live oak (*Quercus virginiana*) and hackberry (*Celtis* spp.), as well as toothache tree (*Aralia spinosa*), red mulberry (*Morus rubra*), and honey locust (*Gleditsia triacanthos*). Shrubs include yaupon (*Ilex vomitoria*), sweet acacia (*Acacia farnesiana*), palmetto (*Sabal* spp.), honeysuckle (*Lonicera* spp.), poison ivy (*Toxicodendron radicans*), and greenbrier vines (*Smilax* spp.).

Bird capture

Birds were captured ($n = 2064$) using nylon mist nets. Captured birds were carefully removed from the nets, and age and sex were determined, when possible (Pyle, 1997). Subcutaneous fat was assessed (Helms and Drury, 1960), each bird was weighed to the nearest 0.1 gram, and a U.S. Fish and Wildlife Service aluminum band was attached. All studies with animals were performed in accordance with a protocol approved by the Institutional Animal Care and Use Committee (IACUC) at the University of Southern Mississippi. An effort was made to sample 10 focal species of migratory birds for ticks based on "foraging cohorts" used to describe standard feeding locations (Barrow et al., 2000). The sub-canopy/canopy group consisted of white-eyed vireos (*Vireo griseus*), red-eyed vireos (*Vireo olivaceus*), and scarlet and summer tanagers (*Piranga olivacea* and *P. rubra*). The understory group included hooded warblers (*Wilsonia citrina*), gray catbirds (*Dumetella carolinensis*), and yellow-breasted chats (*Icteria virens*). The ground group consisted of wood thrush (*Hylocichla mustelina*), northern waterthrush (*Seiurus noveboracensis*), and Swainson's thrush (*Catharus ustulatus*).

Ticks and bird blood collection

Birds were examined for attached ticks by blowing on the feathers of the head, neck, breast, and lower ventral surfaces, thereby separating the feathers, and by examining the nape, bill, eyes, wings, and cloaca (Gregoire et al., 2002). When necessary, feathers were parted using forceps or a fine-toothed comb (Kinsey et al., 2000). When a tick was found, it was carefully removed using fine-tipped forceps and placed in 70% ethanol. The bird species name, band number, tick attachment site, and collection date were documented. Blood samples were taken from the 10 focal species regardless of the presence or absence of ticks to investigate whether tick-borne spotted fever group rickettsiae were present.

Dragging

Dragging was performed for standardized amounts of time during 2009–2010 springs to estimate the relative abundance of ticks at study sites by the drag-cloth method (Falco and Fish, 1988).

Morphological tick identification

Collected ticks were identified using the standard morphological keys (Cooley, 1946; Clifford et al., 1961; Keirans and Durden, 1998). Because half of the world's *Amblyomma* species occur in the Neotropics (Guglielmone et al., 2010) and the preimaginal stages (larva, nymph) of many of these species remain unknown or undescribed, it was often impossible to identify specimens below the genus level.

DNA extraction and nested PCR amplification of spotted fever group rickettsiae

Genomic DNA was extracted individually from ticks and from bird blood samples using the DNeasy blood and tissue kit (Qiagen, CA), following the manufacturer's protocol for isolation of DNA from animal blood samples. Ticks in 70% ethanol were individually cut into small pieces with a scalpel and homogenized in 200 μL of phosphate buffered saline with a sterile micropestle. The homogenized individual tick tissues were disrupted by passage through a 27-gauge needle attached to a 1 mL syringe. The extracted genomic DNA was quantified spectrophotometrically using a Nanodrop-100 (Nanodrop Technologies). Identical amounts of tick DNA and bird blood DNA (~25 ng/μl) were used for each PCR reaction (Roux et al., 1996). Spotted fever group rickettsial infection was detected by using rickettsial outer membrane protein A (*rompA*) gene-specific primers in a nested PCR assay (Blair et al., 2004). Briefly, the primers RR190-70 (5′-ATGGCGAATATTTCTCCAAAA-3′) and RR190-701 (5′-GTTCCGTTAATGGCAGCATCT-3′) were used for the primary reaction, and primers 190-FN1 (5′-AAGCAATACAACAAGGTC-3′) and 190-RN1 (5′-TGACAGTTATTATACCTC-3′) were used for the nested reaction. In the primary reaction, 2.5 μL of DNA template (~62.5 ng) was added to 12.5 μL of 2× PCR Master Mix (Promega, Madison, WI), 8 μL of nuclease-free water, and 1 μL of each primer (10 μM). In the nested reaction, 12.5 μL of 2× PCR Master Mix was used, 8 μL of nuclease-free water, 1 μL of each nested primer (10 μM), and 2.5 μL of the primary PCR reaction. The PCR reactions were performed in a MyCycler Thermal Cycler (Bio-Rad Laboratories, USA) as follows: 1 cycle at 95 °C for 3 min, 35 cycles of 95 °C for 20 s, 46 °C for 30 s, and 63 °C for 60 s, and 1 cycle at 72 °C for 7 min. For each reaction, 2 negative controls (H_2O and PCR reaction minus gene-specific primers) and one positive control (50 ng of *Rickettsia* endosymbiont of *A. maculatum ompA*, GenBank # JX134638) were included. The amplicons were analyzed with a 2% agarose gel containing ethidium bromide and observed using a UV transilluminator. After electrophoresis, PCR products of 540 bp were excised from the gel, and DNA was extracted using a QIAquick DNA gel extraction kit (Qiagen, CA). The purified DNA samples were sent for sequencing to Eurofins MWG Operon (Huntsville, AL). Initially, we sequenced only one strand of each product, but when base calls were dubious, the second strand was also sequenced for comparison. Partial sequences obtained were submitted for BLAST analysis (Altschul et al., 1990) to determine similarities with other spotted fever group rickettsia species.