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SHORT COMMUNICATIONS

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Investigation of a Symptomatic Tick Bite Patient Confirms *Borrelia burgdorferi* in *Ixodes scapularis* and White-Footed Mice in Ashe County, North Carolina

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Abstract

In June 2021, a traveler to Ashe County, North Carolina, was bitten by an *Ixodes scapularis* tick. The patient experienced axillary lymphadenopathy and an erythematous rash near the bite site. We confirmed *Borrelia burgdorferi sensu* stricto through PCR testing and DNA sequencing in the attached tick and later from mice trapped inside the cabin where the patient stayed.

Keywords: Lyme disease, *Borrelia burgdorferi*, *Ixodes scapularis*, North Carolina

Introduction

IXODES SCAPULARIS TICKS infected with *Borrelia burgdorferi* have been present for decades along North Carolina's Atlantic Coast (Levine et al, 2017). However, as recently as the mid-1980s, no *I. scapularis* were found on deer in the Piedmont or mountains (Apperson et al, 1990). In 2014, Herrin et al documented *I. scapularis* in southwestern Virginia, and in 2015 Lantos et al predicted expansion of this tick species into the North Carolina mountains.

The Case Patient

Human case data for the study were obtained per University of North Florida Institutional Review Board approval #468310-9. On June 9, 2021, a 79-year-old woman visiting Ashe County, North Carolina, United States, found a partially engorged tick attached to her right wrist (Fig. 1A) a few days after she arrived. She removed and photographed the tick (Fig. 1B). The next morning, she noticed a tender lymph node in her right axilla and sought medical care from her primary care provider through her patient messaging service.

The provider offered a single-dose doxycycline prophylactic treatment, but refused additional treatment. The patient left the following day to seek further evaluation near her home 4 h away. That same morning, the patient noticed a noncontiguous area of erythema above and slightly distal to the bite site (Fig. 1C). The soonest the patient could find an urgent care center that would see her was June 12 in her Piedmont NC home county. That provider prescribed 2 weeks of 100 mg doxycycline twice a day. No blood specimens were collected for testing. The patient's rash and tender lymph node abated within a few days, and she recovered without further incident.

The tick from the patient was sent to the research laboratory of one of the authors (K.L.C.) and confirmed through standard key (Durden and Keirans, 1996) as a nymph *I. scapularis*. DNA was extracted from the specimen using a standard kit (EZNA Tissue Kit; Omega Biotek, Norcross, GA, USA), and tested through PCR for *Borrelia* species 16S-23S intergenic spacer (IGS) (Bunikis et al, 2004), *Borrelia* species *flaB* gene (Barbour et al, 1996), and *B. burgdorferi ospC* (Qui et al, 2002). In addition, the tick was tested for *Anaplasma phagocytophilum msp2*, *Bartonella* species 16S-23S IGS, *Babesia* species 18S ribosomal rRNA gene, and *Rickettsia* species 23S-5S ribosomal rRNA IGS, using published protocols (Casati et al, 2016; Diniz et al, 2007; Kakumanu et al, 2016; Massung and Slater, 2003).

The attached tick was positive only for *Borrelia* species DNA, and the common *Rickettsia* endosymbiont of *I. scapularis* (*R. buchneri* sequence not submitted to Genbank). The 923 nucleotide (nt) *Borrelia* 16S-23S IGS sequence (GenBank acc. no. OK441075) was most similar (98.9%) to several *B. burgdorferi* strains recovered from

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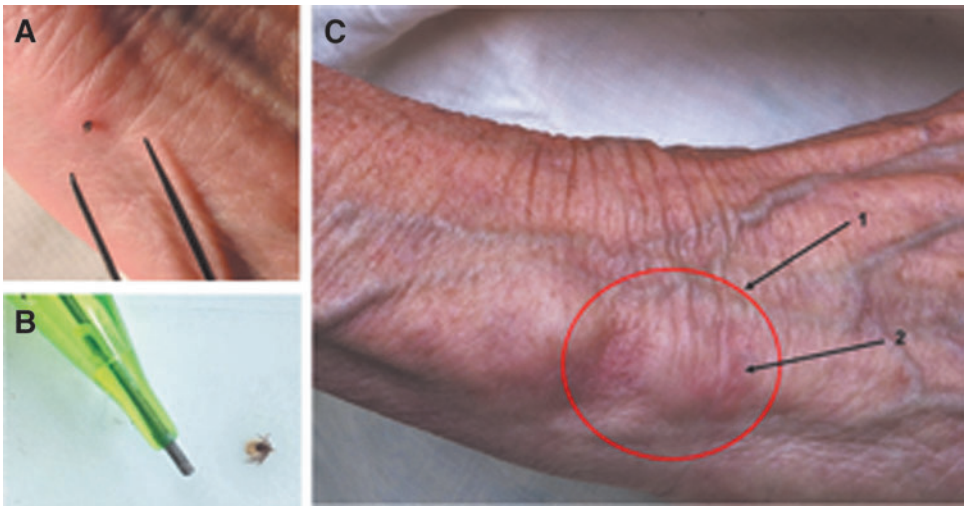

FIG. 1. **(A)** *Ixodes scapularis* nymph attached to patient's wrist before removal. **(B)** Image of the tick after removal by the patient. **(C)** Erythematous lesion near the site of the attached tick. (1) *Circled area* of erythema. (2) Site of tick attachment.

I. pacificus nymphs in California (*e.g.*, GenBank acc. no. JQ308249). The 618-nt *Borrelia flaB* sequence (GenBank acc. no. OK513240) was 99.7% similar (2-nt mismatches) to *B. burgdorferi* strain N40 (GenBank acc. no. CP002228) and 99.5% similar (3-nt mismatches) to strain B31 (GenBank acc. no. CP019767).

In addition, the 530-nt *B. burgdorferi sensu* lato *ospC* sequence derived from the tick (GenBank acc. no. OK513241) was 99.1% similar (5-nt mismatches) to strain B31 (GenBank acc. no. U01894), confirming the presence of a *B. burgdorferi sensu* stricto strain similar to human pathogenic strains commonly found in the northeastern United States.

We made a follow-up visit in August 2022 to the location that the patient visited in 2021. We collected three host-seeking *I. scapularis* nymphs and 184 larvae on a cloth drag in ~1.5 h. Because evidence of rodents was present inside the cabin where the patient had previously stayed, the cabin owner had snap traps set, and trapped four *Peromyscus leucopus* mice, which were provided to the authors for study. Ear tissues, along with 1 additional *I. scapularis* nymph and 57 larvae, were collected from the mice. DNA extracted from the ear tissues and questing nymph ticks was tested by PCR for the aforementioned pathogen groups, but using only the *flaB* gene PCR for *Borrelia* spp.

The three questing ticks were all positive for *R. buchneri* (sequence data not submitted to GenBank), negative for *Borrelia* and *Bartonella*, and one was positive for *Babesia odocoilei* (GenBank acc. no. OP684130). All mice were negative for *Bartonella* and *Babesia* species. Three of the four mice were positive for *B. burgdorferi flaB* gene DNA (GenBank acc. nos. OP689712–OP689714). One mouse-derived *B. burgdorferi flaB* sequence differed from the patient's tick-derived sequence by 1 nt, one differed by 2 nt, and one by 3 nt. In addition, one mouse tested positive for a spotted fever group *Rickettsia* species (GenBank acc. no. OP689711) of unknown pathogenicity.

Discussion

I. scapularis and *B. burgdorferi* have long been present in North Carolina's Atlantic coastal region (Levine et al, 2017), but were uncommon in the more central and western regions of the state (Apperson et al, 1990). More recent studies documented expansion of *I. scapularis* southwesterly along the Appalachian Mountain range (Herrin et al, 2014; Lantos et al, 2015). Hickling et al (2018) showed that *I. scapularis* were either established or present in several counties in eastern Tennessee along the northwest border of North Carolina.

A serological survey of *B. burgdorferi* antibodies in dogs suggested a lack of transmission to dogs in North Carolina before 2005 (Duncan et al, 2004). A later survey of veterinarians (Pultorak and Breitschwerdt, 2014) revealed antibody reactivity in dogs across the state, with reported reactivity statistically greater in the northern half of the state. A recent investigation of several cases of Lyme disease in Buncombe County, NC, identified four human cases associated with an outdoor camp (Barbarin et al, 2020).

The rate of *B. burgdorferi* infection in host-seeking nymph *I. scapularis* at that site was 17%, higher than that documented from questing ticks at most sites studied along the Outer Banks, for comparison (Levine et al, 2017). Together, these findings suggest that *I. scapularis* and *B. burgdorferi* have moved southward into North Carolina, and this southward expansion may not be restricted to the Appalachian corridor.

In the present case, the patient's clinical and epidemiological evidence suggest possible Lyme disease infection. Although the rash at the tick bite site did not appear as an obvious erythema migrans (EM), it is well documented that EM rashes can be highly variable and do not always occur in patients with documented Lyme disease (Rebman et al, 2021). The unequivocal molecular evidence of *B. burgdorferi* in the attached tick demonstrates that this patient was exposed to *B. burgdorferi*.

The additional evidence of several hundred ticks recovered at the site where the patient encountered the infected tick, and *B. burgdorferi* infection in three of four mice trapped inside the cabin where the patient stayed, strongly suggest that *I. scapularis* and *B. burgdorferi* are established at that site in Ashe County. This case did not meet the criteria of a confirmed case of Lyme disease by the North Carolina surveillance case definition (https://epi.dph.ncdhhs.gov/cd/lhds/manuals/cd/casedefs/LYME_DISEASE_CD_2022.pdf). However, this experience demonstrates how confirmed number of cases may underestimate actual Lyme disease exposure risk in an area.

The mountain region of western North Carolina is a popular destination for travelers. In 2019, 26% of North Carolina visitors traveled to that region of the state, totaling ~12.7

million person-trips or ~10.4 million overnight person-trips. Travel to the North Carolina mountains was greatest during the spring and summer (2019 North Carolina Regional Visitor Profile: https://partners.visitnc.com/contents/sdownload/71940/file/2019-North-Carolina-Regional-Visitor-Profile.pdf). The majority (~58%) of travel to that region of the state occurs during spring and summer months, when *I. scapularis* nymph stage ticks are most active.

Conclusion

This report confirms *B. burgdorferi sensu* stricto in an *I. scapularis* acquired by a patient with Lyme disease-like signs and symptoms in Ashe County, North Carolina, and presents the first known DNA sequence evidence of *B. burgdorferi* in ticks and small mammals in Ashe County. Data obtained from the follow-up investigation 1 year later suggest that *I. scapularis* is established, and *B. burgdorferi* is endemic in natural hosts, at the site.

It is likely that risk for Lyme disease is increasing and still underrecognized in this area of North Carolina, and perhaps the entire northern portion of the state. Residents and travelers need to be aware of the risk for Lyme disease in North Carolina and take appropriate tick bite prevention measures. Furthermore, health care providers in North Carolina need to be educated on accurate diagnosis and appropriate treatment of Lyme disease.

Authors' Contributions

K.L.C. and M.H.-G. collected and identified ticks, drafted and edited the article, and created the figure/images. K.L.C. conducted PCR testing and DNA sequence analysis. All authors have seen, approved, and accept full responsibility for the content, and have full access to the data and analyses, as well as drafting and editing the article.

Author Disclosure Statement

No competing financial interests exist.

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