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Microbiota of field-collected *Ixodes scapularis* and *Dermacentor variabilis* from eastern and southern Ontario, Canada

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ABSTRACT

The microbiota of hard ticks has been an area of growing interest due to the potential role that the broader microbial community may play in pathogen carriage and transmission. In the last two decades, Ontario, Canada has experienced rapid changes in the risk of tick-borne disease, primarily due to the range expansion of *Ixodes scapularis*. Another human-biter, *Dermacentor variabilis*, is a longstanding resident of the province, but currently poses minimal risk of pathogen transmission. To examine the microbiota of these two species, we collected adult and nymphal *I. scapularis* and *D. variabilis* from the eastern and southern regions of the province via tick dragging, and conducted next generation sequencing of 19 samples (composed of 45 ticks) via Illumina MiSeq. A total of 1400469 sequences were detected (median 69118/sample; range 23350–155227). The most abundant families of bacteria were unclassified Clostridiales and Ruminococcaceae for both *I. scapularis* and *D. variabilis*. No significant differences in the relative abundances of any phylum, class, order, family or genus were detected between locations (east vs south), sex, life stage or tick species. There were no differences in community membership or structure based on unifrac and AMOVA analyses. Female and male ticks had lower microbial diversity when compared to nymphs, based on the Simpson's index and Shannon evenness index. The findings of our study differ from previous studies of these tick species conducted in other geographic areas, and highlight the potential role geography and related ecological factors may have in shaping the tick microbiota.

1. Introduction

Tick-borne diseases are an emerging public health issue in Ontario, Canada. The blacklegged tick, *Ixodes scapularis*, has undergone significant range expansion in the last two decades (Clow et al., 2016; Ogden et al., 2014). This tick is the vector for numerous pathogens in northeastern North America, including *Borrelia burgdorferi* sensu stricto, the primary causative agent of Lyme disease (Burgdorfer et al., 1982), and its emergence has coincided with a dramatic increase in human cases in Canada (Ogden et al., 2015).

Numerous other tick species exist in the province, but currently pose minimal public health risk (Clow et al., 2016; Lindquist et al., 2016; Nelder et al., 2014). For example, the American dog tick, *Dermacentor variabilis*, has long been established in the province. Although it can transmit the bacteria that causes Rocky Mountain Spotted Fever, *Rickettsia rickettsii*, as well as other pathogens that pose a risk to human and animal health, the infection prevalence of these pathogens in *D. variabilis* is very low in Ontario (Artsob et al., 1984; Scholten, 1977; Wood et al., 2016).

Surveillance programs within the province focus on detecting potential pathogens (Nelder et al., 2014). Although understanding the distribution of pathogens is an important aspect for determining public health risk, it does not provide a complete picture of the microbial community of the ticks (Andreotti et al., 2011; Clay and Fuqua, 2010). Recent studies on various species of Ixodidae have demonstrated that hard ticks have a large population of resident microbes, and that the characteristics of the broader microbial community can influence pathogen acquisition, establishment and transmission, as well as tick reproductive fitness and physiological processes (Ahantarig et al., 2013; Clay and Fuqua, 2010; Narasimhan et al., 2014).

Both *I. scapularis* and *D. variabilis* are obligate parasites. They feed once per life stage on a variety of mammalian species, with the remaining majority of their life spent off-host in the environment (Zolnik et al., 2016). A portion of their microbiota is transmitted vertically, and it is hypothesized that these organisms are primary symbionts required for survival (Ahantarig et al., 2013; Clayton et al., 2015; Moreno et al., 2006). The microbiota then evolves throughout a tick's life, and may be influenced to varying degrees by the environment, blood meals and

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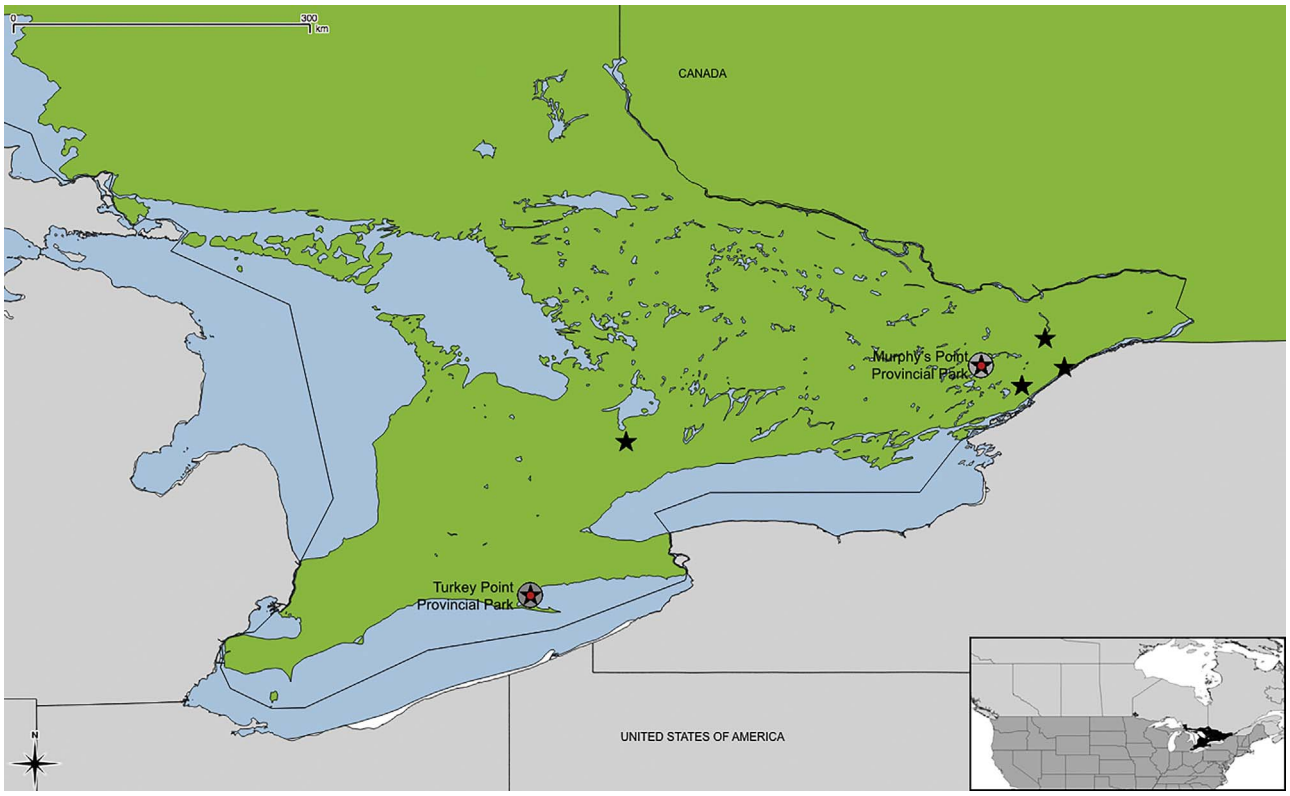

Fig. 1. *Ixodes scapularis* (grey circles) and *Dermacentor variabilis* (black stars) were collected from eastern and southern regions of the province of Ontario, Canada. Turkey Point Provincial Park and Murphy's Point Provincial Park, which are two sites with established *I. scapularis* populations, are provided for reference (red dots). (Data was projected in QGIS 2.18.2 with base layers accessed through the Scholar's Geoportal at the University of Guelph.). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

internal physiological processes (Hawlena et al., 2013; Ryzkiewcz et al., 2016; Zolnik et al., 2016).

We conducted an exploratory study of the microbiota of two tick species of public health interest in Ontario, *I. scapularis* and *D. variabilis*, and compared the characteristics of the microbial communities between species, sex, life stage and geographic location.

2. Methods

2.1. Field sampling

Adult (male and female) and nymphal *I. scapularis* and *D. variabilis* ticks were collected from two geographic areas during the spring and summer of 2014; the first location was in eastern Ontario around Murphy's Point Provincial Park, and the other was in southern Ontario around Turkey Point Provincial Park (Fig. 1). Both provincial parks are endemic areas for *I. scapularis* (Sider et al., 2012). These ticks were collected contemporaneously for other *I. scapularis* research initiatives (i.e., Clow et al., 2016, 2017). Collection of host-seeking (unfed) ticks was conducted using a 1 m^2 flannel drag cloth that was dragged along the forest floor and surrounding vegetation to collect *I. scapularis* and in long grasses and brush to collect *D. variabilis*. All tick samples were placed in 70% ethanol following collection, and stored at room temperature in the laboratory. Ticks were categorized based on species (*D. variabilis* or *I. scapularis*), location (east or south), sex (male or female) and life stage (adult or nymph).

2.2. DNA extraction

All tick samples were rinsed in 70% ethanol followed by sterile distilled water. Whole ticks were then cut and crushed with a sterile scalpel, combined with lysis buffer and shaken on a vortex with glass beads. Following centrifugation, each sample was combined with Proteinase K and then processed using a Maxwell ® 16 kit as per manufacturer's instructions (Promega Corporation, Madison, Wisconsin, USA). Individual *I. scapularis* were small, so pooling was required to provide sufficient sample for DNA extraction. For the pooled samples, between 2–5 *I. scapularis* collected on the same day from the same location, and of the same sex and life stage, were combined.

2.3. Next generation sequencing

Two rounds of PCR were conducted. First, the DNA was amplified by PCR using primers targeting the V4 region of the 16S rRNA gene. The primer set (forward: 5′-AYTGGGYDTAAAGNG-3′ and reverse: 5′-TACNVGGGTATCTAATCC-3′) was designed with overhanging adapters (forward: TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG, reverse: GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG) for annealing to Illumina universal index sequencing adaptors (forward: AATGATACGGCGACCACCGAGATCTACAC-index-TCGTCGGCAGCGTC, reverse: CAAGCAGAAGACGGCATACGAGAT-index-GTCTCGTGGGCTCGG), which were used in the second PCR. Negative controls were used during both PCR runs. Purification of the PCR products were completed with magnetic beads (Agencourt AMPure XP). The final PCR products were evaluated by electrophoresis in 1.5% agarose gel and quantified