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Short communication

Note on *Ehrlichia chaffeensis*, *Ehrlichia ewingii*, and "*Borrelia lonestari*" infection in lone star ticks (Acari: Ixodidae), Nebraska, USA

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ABSTRACT

The lone star tick, *Amblyomma americanum* (L.) (Acari: Ixodidae), is established in southeastern Nebraska yet the prevalence of tick-associated microorganisms is not known. An initial PCR-based analysis for *Ehrlichia chaffeensis*, *Ehrlichia ewingii*, and *Borrelia* infection in host-seeking adult ticks collected in southeast Nebraska was conducted. A total of 251 adult ticks collected in six sites in southeast Nebraska were tested. *E. chaffeensis*, *E. ewingii*, and *Borrelia* spp. were present, and the prevalence of each was approximately 1.6%. This study demonstrates that *Ehrlichia* spp. are present in Nebraska lone star tick populations.

1. Introduction

The lone star tick, *Amblyomma americanum* (L.), is an aggressive ectoparasite and vector of various human and animal pathogens including *Ehrlichia chaffeensis* (human monocytic ehrlichiosis) (Anderson et al., 1993; Ewing et al., 1995), *Ehrlichia ewingii* (canine granulocytic ehrlichiosis) (Anziani et al., 1990; Mixson et al., 2006; Heise et al., 2010), *Rickettsia parkeri* (Tidewater spotted fever) (Macaluso and Azad, 2005; Cohen et al., 2009; Jiang et al., 2012), *Francisella tularensis* (tularemia) (Hopla and Downs, 1953), and the recently described phlebovirus, Heartland virus (Bunyaviridae) (Savage et al., 2013; McMullan et al., 2012). Other microorganisms associated with lone star ticks include *Rickettsia amblyommii* (Apperson et al., 2008; Burgdorfer et al., 1981) and *Borrelia* spp. (Barbour et al., 1996; Stromdahl et al., 2003).

Lone star ticks became established in southeastern Nebraska during the 1980s; presently, populations occur from the south-central to east-central portion of the state, including the Omaha-Lincoln and Platte River corridors (Cortinas and Spomer, 2013). In southeastern collection sites, lone star ticks were more prevalent compared to American dog ticks (*Dermacentor variabilis* (Say)) (Cortinas and Spomer, 2013), and the lone star tick's high densities and wide host range in all life stages likely increase the possibility of encounter with human and animal hosts. Yet, the prevalence of lone star tick-borne pathogens in the state is not known.

Human epidemiological data suggests that lone star tick-borne pathogen transmission may be occurring in the state. There were 17 probable and one confirmed case of human monocytic ehrlichiosis (HME) between 1986 and 1997. These cases were diagnosed by indirect immunofluorescence assay (IFA) at the Centers for Disease Control and Prevention (CDC); at the time, ehrlichiosis cases were not reportable in the state (McQuiston et al., 1999). However, there may have been confounding factors – a lack of tick-borne disease awareness among doctors and the public may have led to under-reporting of locally-acquired cases, some cases may have not been locally-acquired (McQuiston et al., 1999), and cross reactivity of *E. chaffeensis* serological tests with *E. ewingii* and *Anaplasma phagocytophilia* was possible (Paddock and Childs, 2003). Since 1999, ehrlichiosis cases have been reportable, and prior to 2007, no cases were reported. However, between 2007 and 2012, ten cases have been reported and confirmed (Adams et al., 2014; Nieves et al., 2009). These data, coupled with anecdotes from the public about the occurrence of bull's eye rashes, encouraged us to evaluate the prevalence of *E. chaffeensis*, *E. ewingii*, and *Borrelia* spp. in lone star tick adults collected in southeastern Nebraska.

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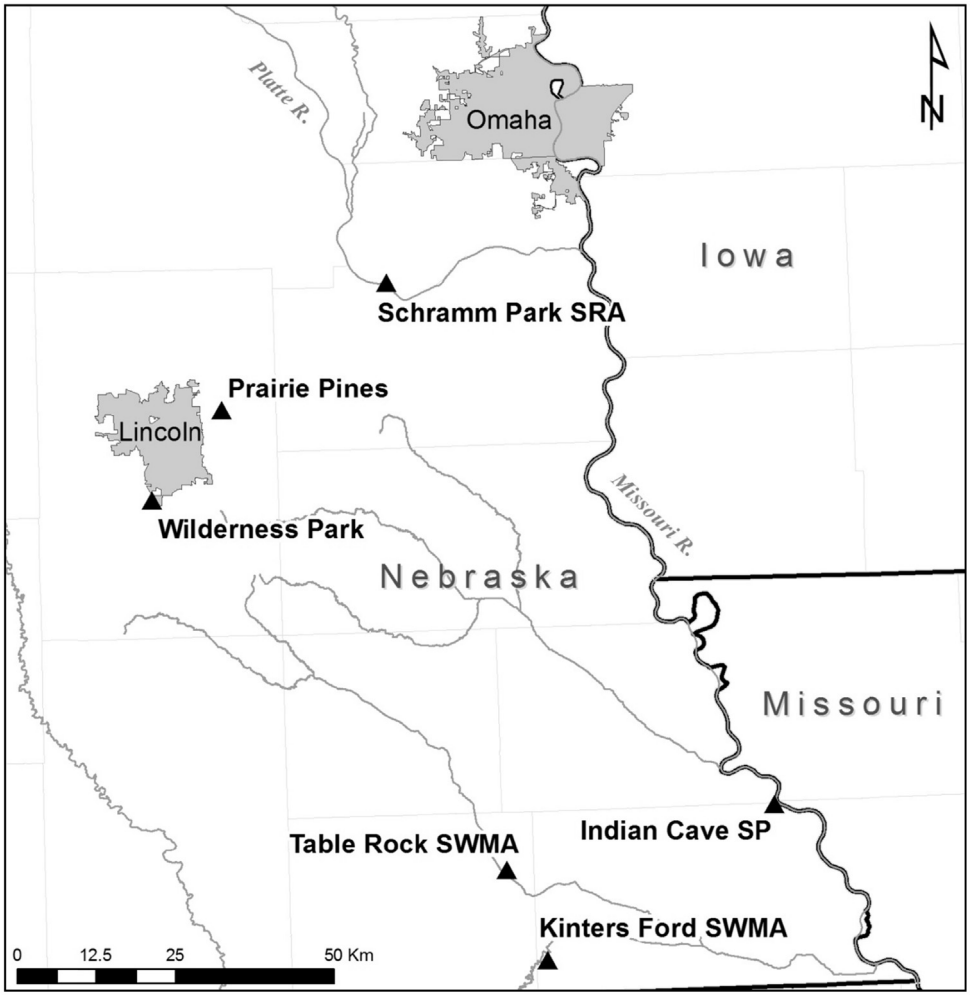

Fig. 1. Map of southeast Nebraska indicating the location of the six collection sites.

2. Material and methods

Ticks were collected from May to August 2012, during the time when lone star tick adults and nymphs are actively questing in the region (Kollars et al., 2000; Cortinas and Spomer, 2013). Six sampling sites where lone star ticks are present were selected (Cortinas and Spomer, 2013). Indian Cave State Park (SP), Kinters Ford State Wildlife Management Area (SWMA), and Table Rock State Wildlife Management Area (SWMA) are located in extreme southeast Nebraska near the Missouri and Kansas borders (Fig. 1). These sites had the highest lone star tick densities in previous collections (Cortinas and Spomer, 2013). Prairie Pines, Schramm Park State Recreation Area (SRA), and Wilderness Park are sites located in the Lincoln–Omaha corridor (Fig. 1).

Indian Cave SP, Prairie Pines, Schramm Park SRA, and Wilderness Park were visited six times every other week, whereas Table Rock and Kinters Ford were visited three times on visits that were at least four weeks apart due to poor weather conditions for sampling. Ticks were collected using carbon dioxide (CO_2) baited traps as previously described (Lockhart et al., 1997). At least 20 traps were used per site visit and traps were placed approximately 20 m apart in forested areas. Traps operated for at least two hours from mid-morning to late-morning, after which the traps were gathered and transported to the laboratory. Ticks were removed from the traps, identified to life stage, sex, and species (Cooley and Kohls, 1944; Keirans and Litwak, 1989; Keirans and Durden, 1998), surface sterilized by being placed in a 1.7 ml centrifuge tube containing 100% ethyl alcohol and vortexed for 5 min, transferred into vials containing 95% ethyl alcohol to maintain tick surface sterility, and kept at −20 °C prior to DNA extraction. Adult ticks were individually stored and nymphs were stored in groups of 10 based on collection site, date, and trap number. Voucher tick specimens were submitted to the Harold W. Manter Laboratory of Parasitology (University of Nebraska State Museum, Lincoln, NE) (Collection numbers 64578, 64579, 64580, 64581).

Individual adult ticks in autoclaved 1.7 mL microcentrifuge tubes were snap frozen in liquid nitrogen and mechanically crushed using two inch disposable pestles (Fisher Scientific, Pittsburgh, PA). Enzymatic digestion was performed with 20 μL of proteinase K and 4 μL polyacryl carrier (Molecular Research Center, Inc., Cincinnati, OH) to isolate small amounts of extracted DNA. Tick DNA was then extracted using the Animal Tissues Spin-Column protocol from DNeasy Blood and Tissue Kit (Qiagen, Valencia, CA). Extraction material from each tick was stored at −20 °C.

Amplification of a ~389 bp fragment of the 16S rDNA fragment for *E. chaffeensis* (Anderson et al., 1992) was performed by combining individual tick extractions into pools of three using a nested PCR protocol. The initial amplification utilized general primers ECB and ECC for *Ehrlichia* spp. (Heise et al., 2010) in a 50 μL reaction. Individual samples contained 25 μL *Taq* PCR Master Mix Kit (Qiagen), 15 μL PCR Grade Water (Roche, Mannheim, Germany), 4 μL of each primer (10 μM) (Integrated DNA Technologies, Coralville, IA), and 2 μL of sample DNA (0.04–55.26 ng/μL). Alternatively, equal amounts of DNA extracted from three ticks (2 μL of sample DNA