Sylvatic Infestation of Oklahoma Reptiles with Immature *Ixodes scapularis* (Acari: Ixodidae)

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ABSTRACT Reptiles were collected in nine counties in Oklahoma from September 2002 to May 2004 and examined for *Ixodes scapularis* (Say) larvae and nymphs to determine seasonal incidence and prevalence of these ticks. In total, 209 reptile specimens consisting of nine species of lizards and seven species of snakes were collected. *Plestiodon fasciatus* (L.) was the most numerous species collected (55%) followed by *Sceloporus undulatus* (Latreille) (17%) and *Scincella lateralis* (Say) (11%). Less than 10 individuals were collected for all remaining reptile species. The infestation prevalence of *I. scapularis* on all reptile specimens collected was 14% for larvae and 25% for nymphs. Larvae were found on lizards from April until September and peaked in May, while nymphs were found from March until September and peaked in April. *I. scapularis* larvae (84%) and nymphs (73%) preferentially attached to the axillae/front leg of *P. fasciatus*. Two chigger species, *Eutrombicula splendens* (Ewing) and *Eutrombicula cinnabaris* (Ewing), were found on 2% of the reptiles collected. No ectoparasites, including ticks, were obtained from the seven species of snakes collected.

KEY WORDS *Ixodes scapularis*, lizard, tick, sylvatic infestation, ectoparasitism

Ixodes scapularis (Say) is the primary vector of the Lyme disease spirochete, *Borrelia burgdorferi* Johnson, Schmid, Hyde, Steigerwalk & Brenner, in the eastern and north-central United States (James and Oliver 1990, Kollars et al. 1999, Casher et al. 2002, Durden et al. 2002, CDC 2014). This disease affects thousands of people every year, primarily in the northeastern and north-central United States (Bertrand and Wilson 1997, CDC 2014). *I. scapularis* is found in virtually the entire eastern one-half of the United States (Dennis et al. 1998), with Oklahoma occupying the western edge of its distribution. Although *I. scapularis* is a competent vector of Lyme disease spirochetes throughout its range (Apperson et al. 1993, Jacobs et al. 2003), the disease is rarely found outside of the northeastern and north-central United States (CDC 2014). A main reason for this disease distribution involves the interaction between competent reservoir hosts of *B. burgdorferi* and the preference of larvae and nymphs (immature life stages) of *I. scapularis* to selectively parasitize these animals (Oliver 1996).

In the northeastern United States, where Lyme disease is hyper endemic, the primary host of immature *I. scapularis* is *Peromyscus leucopus* (Rafinesque) (Oliver et al. 1993b) and other small to medium-sized mammals (Galbe and Oliver 1992, Keirans et al. 1996). These mammals are competent reservoirs of *B. burgdorferi* (Oliver et al. 1993a, Levine et al. 1997, Sonenshine 2014). However, in the southern United States, immature *I. scapularis* are occasionally found on small mammals (Keirans et al. 1996, Durden et al. 2002), but not in sufficient numbers to explain the population of adult ticks found on deer, cattle, and other large mammals. In California, the western black-legged tick, *Ixodes pacificus* Cooley & Kohls, parasitizes the western fence lizard, *Sceloporus occidentalis* Beird & Girard (Schall et al 2000), more than mammals (Casher et al. 2002).

The primary hosts of immature *I. scapularis* in the southeastern United States are not mammals, but reptiles (Goddard 1989, Keirans et al. 1996, Kollars et al. 1999), more specifically, skinks (family Scindidae), fence lizards (genus *Sceloporus*), and glass lizards (genus *Ophisaurus*) (Durden et al. 2002). Specifically, *Plestiodon laticeps* (Schneider) (broad-headed skink), *Plestiodon inexpectatus* and *O. ventralis* are parasitized by immature *I. scapularis* in Georgia (Durden et al. 2002), while *P. laticeps*, *Scincella lateralis* (Lay) (ground skink), and *Aspidoscelis sexlineatus* (L.) (six-lined racerunner) are parasitized by immature *I. scapularis* in North Carolina (Apperson et al. 1993, Levine et al. 1997). All of these reptiles can be found in either wooded areas or ecotones between meadows and wooded areas (Conant and Collins 1998), which is also the optimal habitat for immature *I. scapularis* (Sonenshine 2014).

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While lizard species supporting immature *I. scapularis* have been documented in the southeastern part of the United States, few publications have detailed the hosts on which immature *I. scapularis* feed in the Great Plains region (Koch and Dunn 1980, Kollars et al. 1999, McAllister and Durden 2014, McAllister et al. 2013, 2014). Mainly focusing on tick species on mammals and rodents in eastern Oklahoma, Koch and Dunn (1980) reported immature *I. scapularis* on cottontail rabbits and eastern woodrats with two and three found on fox squirrels and deer mice, respectively. However, *Sceloporus undulatus* (Latreille) (eastern fence lizard) is parasitized by immature *I. scapularis* in Missouri (Kollars et al. 1999). In Oklahoma, McCoy (1995) reported that *Crotaphytus collaris* (Say) (collard lizard) is parasitized by immature *I. scapularis* in two central counties (Major and Comanche). More recently, McAllister et al. (2013, 2014) reported *I. scapularis* nymphs from *Plestiodon fasciatus* (L.), *Scincella lateralis*, and *Sceloporus consobrinus* (McAllister and Durden 2014) captured in one county in southeastern Oklahoma. The aim of this study, therefore, was to characterize the host feeding preferences of immature *I. scapularis* in different regions in Oklahoma, detail the seasonality of activity on reptile hosts, and determine attachment site preference of this medically important tick species on reptiles.

Methods and Materials

Reptile Collections. Reptiles were caught in the field from September 2002 until May 2004. These reptiles were identified using descriptions by Conant and Collins (1998) and examined for attached immature *I. scapularis* and then placed in terraria. Reptiles were caught using one of four different methods: pitfall traps, lizard noose, baiting (Durden and Dotson 1995), or by hand. Pitfall traps consisted of 3.8-liter metal cans placed in the ground with the tops of the cans at ground level. Two small metal fences buried a few centimeters into the ground, each measuring 15.2 cm in height and 6.1 m in length, were stretched from a center can in opposite directions. Another can was placed in the ground at the end of the fences opposite the center can. When the reptiles came in contact with the fence they followed it and eventually fell into one of the three cans. The lizard noose consisted of a long pole, ≥1.8 m in length, with a tension tightening loop made of dental floss attached to the end. When a lizard was spotted, the loop was placed around the lizard's neck and the pole was pulled up to tighten the noose around its neck. Baiting was done using the same pole and noose used in the lizard noose technique but used a live insect such as a cricket, grasshopper, or cockroach tied to the noose as bait. The lizard would seize the insect and stay attached, while it was lifted from the substrate and then lowered into a container. The study protocol was approved through the Institutional Animal Care and Use Committee at Oklahoma State University and adhered to the "Guide for the Care and Use of Laboratory Animals," as prescribed by the Committee on Care and Use of Laboratory Animals of the Institute of Laboratory Animal Resources, National Research Council. The facilities were fully accredited by the American Association of Laboratory Animal Care.

Reptile Habitat and Collection Locale. Reptiles are found in virtually all habitats of Oklahoma including forested areas, around and under logs and rocks, prairies, pastures, in and around bodies of water such as lakes, ponds, streams and rivers, around old buildings such as barns and sheds, and in residential areas. All of these habitats were utilized in our collection efforts for reptiles in the Oklahoma counties of Atoka, Cherokee, Latimer, LeFlore, McCurtain, Osage, Payne, Pushmataha, and Tulsa between September 2002 and May 2004 (Fig. 1).

Reptile Marking and Documentation. Lizards were toe clipped when collected to uniquely mark each individual and assigned an identification number. Snakes were individually kept in Ziploc containers (16 by 24 by 7 cm^3) and assigned an identification number. Reptiles were examined to determine if an individual was a juvenile or an adult. Number of ticks and other mites attached and attachment sites were recorded. Immature ticks were identified using standard guides (Clifford et al. 1961, Durden and Keirans, 1996). Voucher specimens were deposited in the K. C. Emerson Entomology Museum at Oklahoma State University. Chiggers were identified by the late Dr. William J. Wren. Tick attachment sites on reptiles were indicated by marking the location of each tick on an outline drawing of each reptile. Infestation prevalence (percent of infested hosts/total hosts collected), density (number of ticks/number of hosts), and intensity (number of ticks/number of infested hosts) of immature *I. scapularis* were determined for each reptile species (Apperson et al. 1993, Durden et al. 2002).

Statistical Analysis. Attachment preference data of immature *I. scapularis* on several reptile hosts were analyzed using a randomized complete block and a DIFF option in a LSMEANS statement in PROC MIXED (SAS Institute 1996, Cary, NC) with a level of significance of $P < 0.05$.

Results

In total, 219 reptile specimens were collected between September 2002 and May 2004 and examined for immature *I. scapularis* and other ectoparasites. The percent of reptiles collected with each collection method was 79.7% by hand, 15.7% with a lizard noose, 2.8% with pitfall traps, and 1.8% with baiting. These specimens represented nine species of lizards (Table 1) and seven species of snakes from nine counties in Oklahoma (Fig. 1). More *P. fasciatus* individuals were collected ($N = 114$) than all the other reptile species combined ($N = 95$) followed by *S. undulatus* and *S. lateralis* with 35 and 22 individuals, respectively. Less than 10 individuals were collected of all other reptile species. Six lizard species were collected with immature *I. scapularis* attached (Table 1), while three species had no ectoparasites attached (Table 1). No ectoparasites were attached to the 17 specimens of seven species of

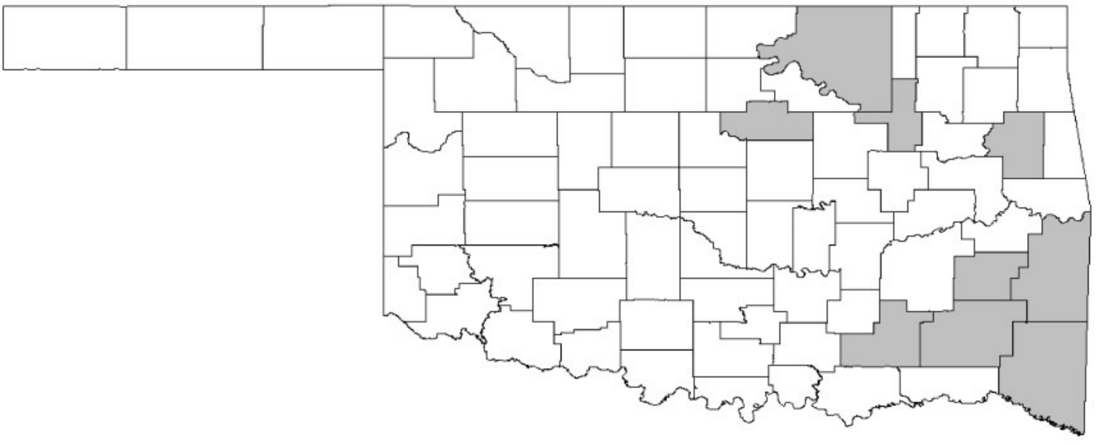

Fig. 1. Counties (shaded) in Oklahoma where reptiles were collected between September 2002 and May 2004.

Table 1. Sylvatic infestation levels of immature *I. scapularis* on nine lizard species in Oklahoma between September 2002 and May 2004

Lizard species	Larvae				Nymphs			
	Prevalence[a] (%) (no. examined)	Density[b]	Intensity[c]	No. attached (range)	Prevalence (%) (no. examined)	Density	Intensity	No. attached (range)
Plestiodon fasciatus	18 (114)	0.7	3.9	82 (1-22)	35 (114)	0.9	2.5	104 (1-13)
Sceloporus undulatus	6 (35)	0.1	2.5	5 (2-3)	14 (35)	0.1	1	5
Scincella lateralis	14 (22)	0.6	2.7	8 (1-5)	18 (22)	0.2	1	4
Plestiodon laticeps	43 (7)	1.7	4	12 (1-9)	43 (7)	1	2.3	7 (1-3)
Aspidoscelis sexlineatus	33 (3)	0.3	1	1	33 (3)	0.7	2	2
Plestiodon obsoletus	0 (3)	0	0	0	0 (3)	0	0	0
Anolis carolinensis	0 (2)	0	0	0	0 (2)	0	0	0
Plestiodon anthracinus	0 (2)	0	0	0	0 (2)	0	0	0
Crotaphytus collaris	100 (1)	4	4	4	0 (1)	0	0	0

[a] Infestation prevalence = percent of infested host/total hosts collected.
[b] Density = number of ticks/number of hosts.
[c] Intensity = number of ticks/number of infested hosts.

snakes collected [*Storeria dekayi* (Holbrook) (brown snake), *Diadophis punctatus* (L.) (ringneck snake), *Tantilla gracilis* Baird & Girard (flathead snake), *Thamnophis proximus* (Say) (ribbon snake), *Coluber constrictor* (L.) (eastern racer), *Tropidoclonion lineatum* (Hallowell) (lined snake), and *Carphophis vermis* (Kennicott) (Western worm snake)].

Infestation of *P. fasciatus* showed 21 of the 114 individuals collected (18%) were infested with a total of 82 *I. scapularis* larvae (Table 1). The density and intensity of larval *I. scapularis* recovered from *P. fasciatus* was 0.7 and 3.9, respectively (Table 1). Forty of the 114 *P. fasciatus* collected (35%) were infested with a total of 104 *I. scapularis* nymphs (Table 1). The density and intensity of nymphal *I. scapularis* recovered from *P. fasciatus* was 0.9 and 2.5, respectively (Table 1). *Plestiodon laticeps*, *A. sexlineatus* and *C. collaris* had a higher infestation prevalence (43, 33, and 100%, respectively) than *P. fasciatus*.

Immature *I. scapularis* parasitized *P. fasciatus*, *S. undulatus*, *S. lateralis*, *P. laticeps*, *A. sexlineatus*, and *C. collaris* between March and September. Larvae were found on lizards between April and September with a peak in host infestation in July (Fig. 2). Nymphs were found between March until September with a two peaks in host infestation in April and July (Fig. 2).

I. scapularis larvae and nymphs were significantly ($P < 0.0001$) more likely to attach to the axilla/front leg region (84% [larvae; $N = 69$] and 73% [nymphs; $N = 76$]) of *P. fasciatus* than other body regions. The next most preferred area of larval attachment was the hind leg/pelvic region with 12% ($N = 10$); followed by the head region (2%; $N = 2$) and mid-body region (1%; $N = 1$). No larvae were found attached in the tail region. *I. scapularis* nymphs were second most likely to be found in the mid-body region with 15% ($N = 16$); followed by the hind leg/pelvic region (6%; $N = 6$) and head region (5%; $N = 4$) and tail region (2%; $N = 1$).

Three reptile species were collected with larval trombiculid mites (chiggers) attached. One specimen of *P. fasciatus* collected in Payne County (July 2003) had a total of 49 chiggers [*Eutrombicula splendens* (Ewing) and *Eutrombicula cinnabaris* (Ewing); previously *alfreddugesi* (Oudemans)] attached in the axilla region. Another specimen of *P. fasciatus* collected in Payne County (July 2003) had five mites attached in a

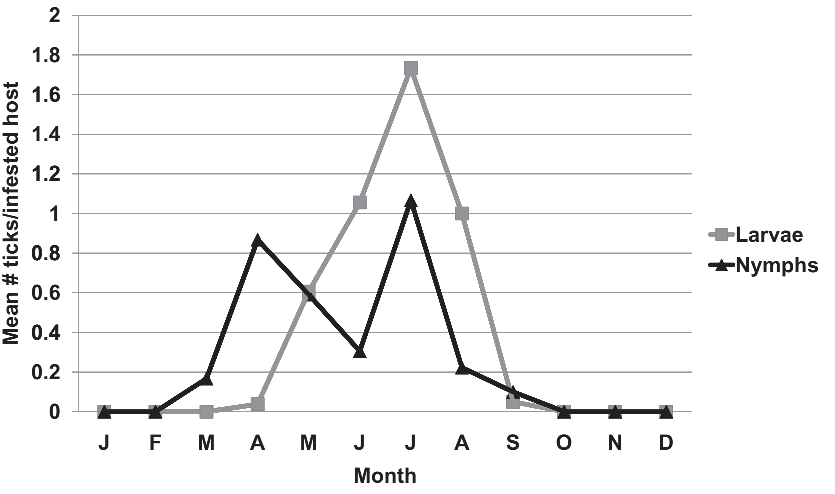

Fig. 2. Seasonal occurrence of *I. scapularis* larvae and nymphs parasitizing lizards collected in Oklahoma between 2002 and 2004. Results are presented as mean number of tick (of each stage) per infested host collected during that particular month.

scar on the dorsal mid-body region but attempts to collect the mites for identification were unsuccessful. One specimen of *Plestiodon obsoletus* (Baird & Girard) (Great Plains skink) collected in Osage County (June 2003) had four chiggers (*E. cinnabaris*) attached in the axilla region. One specimen of *S. lateralis* collected in Payne County (July 2003) had 30 chiggers (*E. splendens*) attached on all four feet.

Discussion

While reptiles, specifically lizards, are known to be the preferential hosts for larval and nymphal *I. scapularis* in the southernmost distribution of this tick species (Goddard 1989, Keirans et al. 1996, Kollars et al. 1999), this study demonstrates the same feeding preference into the western distribution of the tick in the United States (Dennis et al. 1998). In Oklahoma, lizards in the genus *Plestiodon* were the most frequent hosts in the sylvatic infestations. Some of these skinks were previously identified as hosts of immature *I. scapularis* in the southern United States such as *P. fasciatus* (Fitch 1954, Oliver et al. 1993a, Kollars et al. 1999; McAllister et al. 2013), *P. inexpectatus* (Apperson et al. 1993, Levin et al. 1996, Durden et al. 2002), and *P. laticeps* (Apperson et al. 1993, Oliver et al. 1993a, Kollars et al. 1999, Durden et al. 2002).

P. fasciatus was not only the most commonly infested of all the lizard species but was also the most abundant species collected by a ratio of 6:1. Other lizard species found infested with immature *I. scapularis* in this study were also been reported in other studies. These include *S. lateralis* (Apperson et al. 1993, McAllister et al. 2014), *S. undulatus* (Kollars et al. 1999), *S. occidentalis* (Schall et al. 2000, Casher et al. 2002), *C. collaris* (McCoy 1995), and *A. sexlineatus* (Levin et al. 1996; reviewed by McAllister et al. 2013). With the exception of *S. occidentalis* (which does not occur in Oklahoma), these species were also collected in our study with immature *I. scapularis* attached. Immature *I. scapularis* were not found on *Plestiodon anthracinus*, *P. obsoletus*, and *Anolis carolinensis* in the present study which corresponds to previous reports from the southeastern part of the United States (Apperson et al. 1993, Oliver et al. 1993a, Durden et al. 2002).

The results of the current study concur with previous studies that have reported a lack of immature *I. scapularis* on various snake species (Oliver et al. 1993a, Keirans et al. 1996, Durden et al. 2002). Recent studies have demonstrated a possible bias in the kind of snake being caught for these tick host studies with those evaluating pit vipers such as copperheads reporting aggregation by larval *I. scapularis* in the head and cloaca region (Yoder et al. 2013). This behavior appears to be triggered by kairomones that are secreted in the snake's excreta. Future studies focused on host preferences for immature tick species in the Great Plains should try to determine whether pit vipers or other snakes (Corn et al. 2011, Pandit et al. 2011) are also involved in the feeding cycles of immature ticks.

While the activity patterns of *I. scapularis* larvae in Oklahoma were similar to these reported in other studies in the southeastern United States (Durden et al. 2002), nymphal activity was slightly earlier than has been reported in the southeastern region (Apperson et al 1993). Larval *I. scapularis* were found on reptiles as early as April in southeastern Oklahoma. The increase in larval *I. scapularis* observed in May was probably from eggs that were laid in late winter and/or early spring of the same year. The unimodal larval infestation peak in July was later than the peak in May and June reported by Durden et al. (2002) in coastal Georgia.

Nymphs were found to be active as early as March on *P. fasciatus* in southeastern Oklahoma. This differed from reports in the southeastern United States where nymphs are found on lizards mainly between June and September (Apperson et al. 1993). Additionally, the bimodal nymphal infestation peaks in April and July are quite different than that reported in the eastern United States (Apperson et al. 1993, Durden et al. 2002). This might be indicative of a change in host-seeking behavior in May and June but most likely, it was owing to the greater numbers of lizards caught during this period as compared with the other months that brought the infestation rates down. These nymphs probably overwintered as flat (unfed) nymphs and began seeking hosts when temperatures became warm enough for both parasite and host to become active. The preference of *I. scapularis* nymphs to feed primarily on lizards throughout the southern United States may limit the effectiveness of using dragging methods for surveillance of nymph populations (Cilek and Olson 2000, Diuk-Wasser et al. 2006, Goddard and Piesman 2006).

The majority of *I. scapularis* larvae and nymphs were found attached to the axilla region of *P. fasciatus*, the same region where the *E. cinnabaris* (previously *alfreddugesi* Bennett et al. 2014) and *E splendens* mites were attached. This preference to attach in the head region probably has to do with accessibility to blood as owing to less tightly packed scales, protection from desiccation, and a reduced likelihood to be rubbed off as the lizard moves around (Fitch 1954) or it may involve the hygiene habits of lizards that groom with their mouths. While providing an optimal location for immature ticks and mites to feed, studies from *Ixodes ricinus* (L.) on *Psammodromus algirus* lizards suggest that it is also an optimal site that does not interfere with the fitness of the lizard (Salvador et al. 1999).

This study confirms earlier reports of *E. splendens* and *E. cinnabaris* mites on lizards (*S. undulatus* and *C. collaris*) and snakes (*Heterodon platirhinos*, *Masticophis flagellum*, and *Sonora semiannulata*) in Oklahoma (Loomis 1956, Crossley 1960, McCoy 1995). Commonly occurring through the United States (Loomis 1956, Walters et al. 2011), *E. cinnabaris* is commonly found on a wide variety of lizards, snakes (including copperheads and rattlesnakes), frogs, toads, tortoise, and turtles (McCoy 1995, Walters et al. 2011). *E. splendens* also has been reported throughout the United States and has been collected from diverse hosts such as wild-caught Burmese pythons (Corn et al. 2011), white-tailed deer (Forrester et al. 1996), black bear (Cunningham et al. 2001), and humans (Jenkins 1949).

This study demonstrated that, much like the southern and southeastern United States, lizard species in Oklahoma serve as important hosts for immature *I. scapularis*. While highlighting a possible earlier activity pattern of nymphs in Oklahoma than in other places, questions remain regarding host preference. Studies of immature *I. scapularis* on reptile hosts often report geographical differences in infestations between lizard species (Apperson et al 1993, Oliver et al. 1993a, Durden et al. 2002). These differences could possibly be due to small samples sizes or could actually highlight geographical differences in host preference. While immature *I. pacificus* ticks on the west coast of the United States appear to prefer male reptiles (Lumbad et al. 2011, Pollock et al. 2012), further studies are needed to compare infestation rates between development stages of *I. scapularis* and infestation patterns of the same reptiles over time. Most informative would be an evaluation of infestation interactions of immature *I. scapularis* with lizards and rodents in the same habitat in the Great Plains region much like that reported by Eisen et al. (2004) for *I. pacificus* in California.

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