Prevalence and Species of Ticks on Horses in Central Oklahoma

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ABSTRACT Ticks are common on horses, but there is a dearth of contemporary data on infestation prevalence, predominant species, and tick-borne disease agents important in this host. To determine the species of ticks most common on horses and the prevalence of equine exposure to and infection with tick-borne disease agents, ticks and blood samples were collected from 73 horses during May, June, and July of 2010. Adult ticks were identified to species, and antibodies to Ehrlichia spp., Anaplasma spp., and Borrelia burgdorferi were identified using indirect fluorescence antibody assay, a commercial point-of-care enzyme-linked immunosorbent assay, or both. In total, 1,721 ticks were recovered at the majority (85%) of equid examinations. Amblyomma americanum (L.) was the most common tick collected (1,598 out of 1,721; 92.9%) followed by Dermacentor variabilis (Say, 1821) (85 out of 1,721; 4.9%) and Amblyomma maculatum Koch, 1844 (36 out of 1,721; 2.1%); single specimens of Ixodes scapularis Say, 1821 and Dermacentor albipictus (Packard, 1809) were also identified. Antibodies reactive to Ehrlichia spp. were found in 18 out of 73 (24.7%) of horses tested, and were more commonly identified in horses with moderate or high tick infestations than those with low tick infestations (P < 0.001). These data support A. americanum as the most common tick species infesting horses in central Oklahoma from May through July and suggest horses are also commonly exposed to an Ehrlichia sp.

KEY WORDS Amblyomma americanum, Amblyomma maculatum, Dermacentor variabilis, Ehrlichia spp., horse

Ticks are a common arthropod pest of horses (Equus caballus) in North America (Bishop and Trembley 1945). In addition to causing irritation, skin wounds, allergy, and blood loss, some tick species are known to transmit pathogens to horses (Trietschler 1965, Magnarelli et al. 2000, Stiller et al. 2002). Increased interest regarding the importance of equine infections with tick-borne disease agents such as Borrelia burgdorferi and Anaplasma phagocytophilum together with the recent recognition of equine piroplasmosis in horses in the United States (Scoles et al. 2011, Short et al. 2012), have underscored the importance of improving current understanding of the tick species most commonly found on horses.

Although contemporary surveys are lacking, historic data document the presence of Amblyomma spp., Dermacentor spp., Ixodes spp., and Rhipicephalus (Boophilus) spp. ticks on horses in the United States (Bishop and Trembley 1945, Carroll and Schmidtmann 1986, Schmidtmann et al. 1998); Otobius megnini (Dugès), the spinose ear tick, is also a common pest of horses in the western United States (Madigan et al. 1995). Both the number and the geographic distribution of reports of tick species have increased in North America in recent years (Childs and Paddock 2003, Mertins et al. 2010), and infestation pressure on horses has undoubtedly also increased. Products labeled against ticks on horses are available commercially, but horse owners continue to struggle to control these arthropods; indeed, equine tick infestations are commonly seen, and complaints regarding ticks are often heard by both veterinarians and extension agents (S. Little and J. Talley, personal communication).

To better understand the nature and importance of tick infestations on horses, we surveyed horses in north-central Oklahoma to determine the current prevalence of adult tick infestations and the predominant species responsible for those infestations. Owing to a concurrent observation that horses in this region commonly have antibodies to Ehrlichia spp. (Carmichael et al. 2010), we also tested horses for serologic reactivity to Ehrlichia spp. and compared those results with our tick infestation data.

Materials and Methods
Privately owned horses at least 6 mo of age were identified for inclusion in the study through extension

IDEXX Laboratories (Westbrook, ME) donated the 4DX SNAP assays for use in this study at the request of S.E.L., who has received research support and consulting fees from IDEXX Laboratories in the past 5 yr. 1 Department of Veterinary Pathobiology, Center for Veterinary Health Sciences, Oklahoma State University, Stillwater, OK 74078. 2 Department of Veterinary Clinical Sciences, Oklahoma State University, Stillwater, OK 74078. 3 Department of Entomology and Plant Pathology, Oklahoma State University, Stillwater, OK 74078. 4 Corresponding author, e-mail: susan.little@okstate.edu.
entomologists and clinicians at the Boren Veterinary Medical Teaching Hospital at Oklahoma State University. Horses with a history of tick infestation, frequent exposure to tick-infested habitat, or both, were selected for inclusion. All study protocols were approved through the Institutional Animal Care and Use Committee at Oklahoma State University. Sample collections occurred from May to July of 2010. At enrollment and approximately every 2 wk thereafter, each horse was examined for ticks, and all adult ticks identified were removed and placed into 70% ethanol. Immature ticks, when present, were also occasionally collected but were not enumerated or identified. Tick examination was conducted by close visual inspection by two individuals standing on either side of the horse, beginning at the head and neck and proceeding systematically down the mane along the dorsal midline, across the dorsum and lateral thorax caudally, until the tail was reached. After thorough examination of the tail and perianal region, inspection continued with examination of each foreleg, axillary region, ventrum, hindlegs, and inguinal region. Ticks were collected from the face and external ears when visible. However, horses were not sedated and thus inspection and collection of ticks from the internal ear canals was not performed.

Whole blood (10 ml) was collected from each horse at each examination via jugular venipuncture directly into vacuum tubes containing ethylenediaminetetraacetic acid (EDTA) or no anticoagulant. After blood was allowed to clot at room temperature, serum was harvested by centrifugation at 1,500 × g and stored frozen at −20°C. An aliquot of EDTA-anticoagulated whole blood was tested for antibodies to B. burgdorferi, Ehrlichia spp., and Anaplasma spp. using a commercial enzyme-linked immunosorbent assay (ELISA; 4DX SNAP, IDEXX Laboratories, Westbrook, ME) according to manufacturer’s instructions, and the remainder held frozen at −20°C. Adults ticks were enumerated, the sex identified, and the species determined with microscopic examination and comparison with standard keys (Keirans and Litwak 1989).

Serum samples were assayed via indirect fluorescence antibody (IFA) testing for antibodies reactive to Ehrlichia chaffeensis as previously described (Dawson et al. 1991). Briefly, E. chaffeensis (Arkansas strain) infected DH52 cells suspended in culture medium (Minimum Essential Medium, HyClone Laboratories, Logan, UT) were adhered to each well of a 12-well Teflon-coated slide, allowed to air dry for 4 h, fixed in acetone for 15 min, allowed to air dry for 30 min, and then held at −70°C until use. Slides were thawed immediately before use. Each serum sample was screened at 1:64 and then successive twofold dilutions of positive sera were evaluated; detection of bound antibodies was achieved using FITC-conjugated anti-horse IgG (Kirkegaard and Perry Laboratories Inc., Gaithersburg, MD) diluted 1:250 in 0.01 M phosphate-buffered saline (PBS). The maximum titer was reported as the highest dilution at which specific fluorescence was observed.

**Table 1. Species, sex, prevalence, and intensity of adult tick infestations on horses**

<table>
<thead>
<tr>
<th>Species</th>
<th>No. (%)</th>
<th>M:F Prevalence (%)</th>
<th>Geometric mean intensity (range)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A. americanum</td>
<td>1,598 (92.9%)</td>
<td>917:681</td>
<td>64/73 (87.6%)</td>
</tr>
<tr>
<td>A. maculatum</td>
<td>36 (2.1%)</td>
<td>23:13</td>
<td>19/73 (26.0%)</td>
</tr>
<tr>
<td>D. variabilis</td>
<td>55 (4.9%)</td>
<td>36:49</td>
<td>27/73 (37.0%)</td>
</tr>
<tr>
<td>I. scapularis</td>
<td>1 (&lt;0.1%)</td>
<td>0:1</td>
<td>1/73</td>
</tr>
<tr>
<td>D. albipictus</td>
<td>1 (&lt;0.1%)</td>
<td>1/73</td>
<td>1.0</td>
</tr>
<tr>
<td>Total</td>
<td>1,721</td>
<td>977:744</td>
<td></td>
</tr>
</tbody>
</table>

Chi-square analysis with significance assigned at P < 0.05 was used to compare age and gender of infested horses and prevalence of antibodies reactive to *Ehrlichia* spp. to intensity of tick infestation on horses.

**Results and Discussion**

In total, 73 horses (17 males and 56 females) were examined in this study. Age, as provided by owners, ranged from 6 mo to 34 yr (average 10.6 yr). Ages were not provided for five of the horses enrolled in the study. Adult ticks were identified on 67 out of 73 (91.8%) horses. *Amblyomma americanum* (L.) was the most common tick identified, accounting for 92.9% (1,598 out of 1,721) of all adult ticks collected, and was present on 64 out of 67 (95.5%) horses with tick infestations. Intensity of infestation ranged from 1 to 61 adult ticks (geometric mean 6.5 ticks). Other ticks included *Dermacentor variabilis* (Say, 1821) (55 out of 172; 4.9%) on 29.9% of horses with tick infestations. *Amblyomma maculatum* Koch, 1844 (36 out of 1,721; 2.1%) on 14.3% of horses with tick infestations. Single specimens of *Dermacentor albipictus* (Packard, 1869) and *Ixodes scapularis* Say, 1821 were also recovered (Table 1).

Of infested horses, 14 were males and 53 were females. No significant difference was detected in frequency or intensity of infestation by gender or age of horses compared with the surveyed population as a whole (P > 0.05). A slight shift in species composition was detected over time, with *A. maculatum* and *D. variabilis* accounting for 1.6% (6 out of 369) of all ticks collected from horses in May, but 8.5% (115 out of 1352) of those collected in June and July (P < 0.001). Antibodies reactive to p30/p30–1 of *Ehrlichia canis* were detected by the ELISA assay in 16 out of 73 horses (21.9%). Antibodies reactive to *E. chaffeensis* on IFA were identified in 18 out of 73 horses (24.7%), with titers ranging from 1:64–1:512 (geometric mean of inverse positive titers = 117.4). Discordant results were present in three horses, one of which was positive on ELISA but negative on IFA, and two of which were positive on IFA but negative on ELISA. Antibodies reactive to p30/p30–1 of *E. canis* on ELISA and
to *E. chaffeensis* on IFA were identified in 10.7% (6 out of 56) and 8.9% (5 out of 56), respectively, of horses with <20 *A. americanum* ticks, and in 58.8% (10 out of 17) and 76.5% (13 out of 17), respectively, of horses with ≥20 *A. americanum* ticks (*P* < 0.001). Seroprevalence using both assays was greater in horses with ≥40 *A. americanum*, but sample size limited statistical power and, thus, precluded evaluation of this association. Antibodies to C6 of *B. burgdorferi* and p44/MSP2 of *A. phagocytophilum* were detected in one and three horses, respectively (Table 2).

*A. americanum* was the most common tick identified on horses, consistent with previous reports of ticks from people and deer from the southern United States (Merten and Durden 2000, Paddock and Yabsley 2003). The research was supported through the Krull–Ewing Endowment to S.E.L. at Oklahoma State University.

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### References Cited


### Table 2. Antibodies to *Ehrlichia* sp. on ELISA and IFA in horses with low (0–19 ticks per horse), moderate (20–39 ticks per horse), and high (≥40 ticks per horse) adult *A. americanum* infestations

<table>
<thead>
<tr>
<th>Infestation category</th>
<th>No. of horses</th>
<th>Positive via ELISA</th>
<th>Positive via IFA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low</td>
<td>56</td>
<td>6/56 (10.7%)</td>
<td>5/56 (9.9%)</td>
</tr>
<tr>
<td>Moderate</td>
<td>9</td>
<td>5/9 (55.6%)</td>
<td>6/9 (66.7%)</td>
</tr>
<tr>
<td>High</td>
<td>8</td>
<td>5/8 (62.5%)</td>
<td>7/8 (87.5%)</td>
</tr>
<tr>
<td>Total</td>
<td>73</td>
<td>16/73 (21.9%)</td>
<td>18/73 (24.7%)</td>
</tr>
</tbody>
</table>


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