Frequency and Distribution of Rickettsiae, Borrelia, and Ehrlichiae Detected in Human-Parasitizing Ticks, Texas, USA

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To describe the presence and distribution of tickborne bacteria and their vectors in Texas, USA, we screened ticks collected from humans during 2008–2014 for *Rickettsia, Borrelia, and Ehrlichia* spp. Thirteen tick species were identified, and 23% of ticks carried bacterial DNA from at least 1 of the 3 genera tested.

Ticks are vectors for a variety of microorganisms, many of which are known agents of zoonotic disease. Although much current research is focused on areas where these diseases are common, it is crucial to collect data from areas with fewer diagnoses of tickborne illness. In Texas, USA, tickborne diseases caused by *Rickettsia, Borrelia,* and *Ehrlichia* bacteria are diagnosed less frequently than in some areas of the United States (1); however, those agents have been documented to occur (2), and many medically relevant tick species, capable of carrying and transmitting these pathogens, are established in various geographic areas of Texas (1). Long-term surveillance data encompassing consecutive seasons and a wide geographic range are necessary to ascertain disease transmission risks associated temporally or geographically with established or emerging tickborne pathogens and their vectors. The University of North Texas Health Science Center Tick-Borne Disease Research Laboratory (UNTHSC-TBDL), the primary tick-testing facility for Texas Department of State Health Services Zoonosis Control (TX DSHS), receives ticks continually throughout the year. The data collected from this testing provide an assessment of the prevalence of tick species and associated tickborne bacterial agents collected in Texas.

The Study

From October 1, 2008, through September 30, 2014, ticks removed from humans were sent by TX DSHS to UNTHSC-TBDL, where they were tested by using PCR-based methods, then underwent by DNA sequence analysis to determine the presence of *Rickettsia, Borrelia,* and *Ehrlichia* spp. Morphologic identification of tick species was implemented by entomologists at TX DSHS. Ticks that could not be classified morphologically were identified at UNTHSC-TBDL by sequencing mitochondrial 16S rDNA (data not shown).

Each tick was sent to UNTHSC-TBDL in an individual collection tube. Upon arrival, ticks were processed according to the laboratory’s standard protocol, as described by Williamson et al. (2). After bead pulverization, we extracted DNA using the E.Z.N.A. Mollusc DNA Isolation Kit (Omega Bio-Tek, Norcross, GA, USA) following the manufacturer’s protocol.

DNA from each specimen was screened in duplicate by PCR for *Rickettsia, Borrelia,* and *Ehrlichia* spp. as previously described (2) by using primers listed in Table 1. PCR products were evaluated, and presumptive-positive amplifications were purified for sequencing (2). Cycle sequencing reactions were performed in both directions by using BigDye Terminator version 3.1 chemistry (Life Technologies, Carlsbad, CA, USA). Dideoxy chain termination products were detected electrophoretically on an ABI 310 or 3130xL Genetic Analyzer (Life Technologies). Analyzed sequences were compared with reference data in GenBank (http://blast.ncbi.nlm.nih.gov/). Sequences were submitted to GenBank under accession nos. KP861333–KP861347.

The TX DSHS submitted 1,112 ticks to UNTHSC-TBDL during October 1, 2008–September 30, 2014, of which 1,062 originated in Texas. Thirteen tick species were identified; most were *Amblyomma americanum* (55.7%), followed by *Dermacentor variabilis* (15.0%), *Rhipicephalus sanguineus* (13.0%), *Ixodes scapularis* (5.6%), *A. maculatum* (5.4%), and *A. cajennense* (2.9%). Approximately 23.3% of ticks originating in Texas tested positive for DNA from *Rickettsia, Borrelia,* or *Ehrlichia* bacteria (Table 2; online Technical Appendix Table, http://wwwnc.cdc.gov/EID/article/22/2/15-0469-Techapp1.pdf). Of these bacteria, most belonged to spotted fever group rickettsiae (SFGR); *A. americanum* was the most common tick species found to carry an SFGR agent. The most frequent SFGR sequences detected demonstrated
Table 1. Primers used for screening of human-parasitizing tick specimens, Texas, USA, October 1, 2008–September 30, 2014

| Primer name | Gene | Primer sequence, 5′ → 3′ | Specificity | Amplicon, bp | Reference |
|-------------|------|-------------------------|-------------|-------------|-----------|
| Borrelia spp. |      |                         |             |             |           |
| FlaLL       | flaB | ACATATTCCATGGCAGACAGGCTT | Genus 664   | (3)         |           |
| FlaRL       | flaB | GCAAATCAGGCCTTGCAGATTTG  | Genus 664   | (3)         |           |
| FlaLS       | flaB | AAACGCTGAGAGCTGATTTGAG   | Genus 330   | (3)         |           |
| FlaRS       | flaB | CTTGATCATCTATCAATTACAGC  | Genus 196   | (2)         |           |
| BL-Fla 522F | flaB | GTTGCTATTTGCGAGCCAAGAGGG | B. lonestari | 660        | (2)       |
| BL-Fla 1182R| flaB | GCATTGATTGTTGCTGCAATCAGCC | B. lonestari | 198        | (2)       |
| BL-Fla 662F | flaB | CTGAAGAGCTGGAATGCAAGCTCC | B. lonestari | 693        | (2)       |
| BL-Fla 860R | flaB | GAGCTAACTCCACCTGAGCTGG   | B. lonestari | 693        | (2)       |
| BL-16S 227F | 16S  | TCACACTGGAACCTGAGATCGGTC | Genus 693   | (2)         |           |
| BL-16S 920R | 16S  | GAATTAACACCATGCTGCCAGGC  | Genus 693   | (2)         |           |
| Rickettsia spp. |   |                         |             |             |           |
| Rr.190 70P  | rompA| ATGGCGCAATTTTCTCAAAAAA   | Genus 398   | (6)         |           |
| Rr.190 602N | rompA| AGTTGACATTGTCGCTCCTCCCT  | Genus 650   | (5)         |           |
| BG1–21      | rompB| GCGAATTAATAGTGGCTGACGG   | Genus 650   | (5)         |           |
| BG2–20      | rompB| GCATCTGACATAGCCTTTCC     | Genus 650   | (5)         |           |
| Ehrlichia spp. |  |                         |             |             |           |
| Ehr DSB 330F| dsb  | GATGATGTCTGAAGATATGAAAA  | Genus 873   | (7)         |           |
| Ehr DSB 72R | dsb  | CTGCTCTGCTATTTCTTCTTT    | Genus 873   | (7)         |           |
| Ehr map1F   | map1 | ATTATTTACCTGTTGTCGCTTTTCTGA | Genus 873 | (7)         |           |
| Ehr map1R   | map1 | CCTTCCTCAATTCTTCTTCC     | Genus 873   | (7)         |           |
| Ehr Pmap2F  | map1 | GACACCAAGGAGATGATACGGG   | Genus 873   | (7)         |           |
| Ehr Pmap2R  | map1 | CTAAGTCAGTCAATTACCTGAC   | Genus 873   | (7)         |           |
| Tick DNA    |      |                         |             |             |           |
| 16S-1       | mt16S| CCGGTCTGAATCTGAGATCAGAG | Unknown     | 300         | (8)       |
| 16S+2       | mt16S| TGGGCGAAGAGCCCTTATGAA    | Unknown     | 300         | (8)       |

100% identity to Candidatus Rickettsia amblyommii rompA (GenBank accession no. EF194096). Candidatus R. amblyommii was detected in both A. americanum and A. cajennense ticks and showed prevalence rates of 30.3% and 32.3%, respectively. The second most common SFRG rompA sequences were 100% homologous to the previously termed rickettsial I. scapularis endosymbiont, which has been officially named R. buchneri (accession no. KP172259) (9). Five A. maculatum specimens contained DNA sequences identical to R. parkeri rompA (accession no. KC003476). Sequences that shared 100% similarity to 1 specific R. rhipicephali isolate (accession no. U43803) and 99% similarity to other R. rhipicephali rompA isolates (accession nos. EU109175–EU109178) were obtained from 4 D. variabilis ticks. Sequences isolated from 2 D. andersoni ticks were identical to R. peacockii rompA and rompB (accession nos. FM883671 and CP001227, respectively). Tick species was confirmed by sequencing mitochondrial 16S rDNA. Sequences from both specimens aligned 99% with D. andersoni (accession no. EU711343) and 94% with D. variabilis (accession no. L34300). D. andersoni is not known to inhabit Texas (1,10), so this finding could suggest a novel geographic association.

The total prevalence of borreliae detected was 1.1%. DNA sequences sharing 100% identity to B. lonestari were found in 8 A. americanum ticks (1.4%). As seen by Stromdahl et al., the B. lonestari isolates matching sequences in this study depended on the insertion or deletion of a nucleotide triplet, AAG (11). Sequences from 7 tick samples matched 100% with B. lonestari flaB isolates containing the additional triplet (accession no. AY850063), and 1 sequence was identical to B. lonestari flaB isolates lacking the triplet (accession no. AY850064). Of the 8 A. americanum

Table 2. Number of positive bacterial DNA sequences identified for each human-parasitizing tick species, Texas, USA, October 1, 2008–September 30, 2014*

| Tick                  | No. positive |
|-----------------------|--------------|
|                       | Borrelia     | Ehrlichia     | Rickettsia   |
|                       | amblyommi†   | parkeri       | peacockii    |
|                       |              |               |              |
| Amblyomma americanum  |              |               |              |
| A. cajennense         | 0            | 0             | 2            |
| A. maculatum          | 2            | 0             | 0            |
| Dermacentor variabilis| 1            | 0             | 0            |
| D. andersoni          | 0            | 0             | 0            |
| Ixodes scapularis     | 0            | 0             | 0            |
| Rhipicephalus sanguineus | 0         | 0             | 0            |
| Total                 | 3            | 1             | 8            |

*Only tick species originating in Texas that tested positive for Borrelia, Ehrlichia, or Rickettsia spp. by DNA sequence analysis are shown. Additionally, 2 A. maculatum ticks from Texas were positive for Panola Mountain Ehrlichia. UNID, unidentified species.
†Candidatus species.
ticks from which the *B. lonestari* sequences were obtained, 6 were co-infected with *Candidatus* R. amblyommi. DNA extracts from 1 *I. scapularis* tick contained a sequence consistent with *B. burgdorferi* sensu stricto (s.s.) and was co-infected with *R. buchneri*. The *flaB* sequence matched 100% to (accession no. CP002228), and 99% to (accession no. CP009656) *B. burgdorferi* s.s. reference sequences. The *Borrelia* 16S rDNA sequence showed 100% identity to (accession no. CP009656) and differed by 1 single nucleotide polymorphism from (accession no. CP002228) *B. burgdorferi* s.s. reference sequences. The *flaB* sequence matched 100% to (accession no. CP002228), and 99% to (accession no. CP009656) *B. burgdorferi* s.s. reference sequences. The *flaB* gene sequence from 1 *D. variabilis* tick shared 100% identity with *Candidatus* B. *texasensis* (accession no. AF264901). Samples from 2 *A. maculatum* ticks showed *flaB* sequences identical to a novel *Borrelia* sp. Those *flaB* sequences were identical to a novel *Borrelia* sp. (accession no. KF395230) previously found in *A. maculatum* ticks in Mississippi and known to share a phylogenetic clade with *B. turcica* (12). *Borrelia* 16S rDNA primers produced nonspecific amplification with these 2 samples.

Phylogenetic analysis was performed by using MEGA version 5.1 (http://www.megasoftware.net) using GenBank reference sequences to examine relationships between the *Borrelia* sp. from this study, *B. turcica*, and both Lyme disease–associated and relapsing fever borreliae (Figure). The results supported findings by Lee et al. that the novel *Borrelia* sp. *flaB* sequences were more closely related to the reptilian *Borrelia* than the other 2 *Borrelia* groups (12).

Two *A. americanum* ticks contained DNA sharing 100% identity with *Ehrlichia chaffeensis* dsb (accession no. CP000236). One of these ticks was co-infected with *Candidatus* R. amblyommi. Prevalence of *E. chaffeensis* in the *A. americanum* specimens tested was 0.34%. In addition, 2 of 42 *A. maculatum* ticks tested for the emerging pathogen Panola Mountain *Ehrlichia* sp. (PME) (7) each produced a *map1* sequence that was 100% homologous to 2 separate PME reference sequences (accession nos. EU272356, EU272358). These sequences differed from each other by 1 single nucleotide polymorphism. This finding represents a novel association, as *A. americanum* is the known vector for PME (7). A subset of 141 *A. americanum* ticks was also tested for PME, with negative results.

**Conclusions**

Frequency of tickborne zoonoses in Texas remains low compared with some regions of the United States. We report the detection of known pathogens along with bacteria of unknown pathogenicity in human-parasitizing ticks commonly found in Texas. Our findings underscore the importance of better characterization and continued surveillance of the frequency and distribution of tick species and the bacterial agents they carry. Continued monitoring in low-risk areas provides data regarding the presence of potential emerging pathogens and vectors not yet commonly identified, which could pose unidentified threats to public health.

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Technical Appendix

Technical Appendix Table. Summary of number, identity, and bacterial screening results for ticks submitted by Texas Department of State Health Services Zoonosis Control, October 1, 2008–September 30, 2014*

| Tick                    | No. positive/no. tested | Borrelia spp. | Ehrlichia spp. | Rickettsia spp. | Total |
|-------------------------|-------------------------|---------------|----------------|-----------------|-------|
| **Amblyomma americanum** |                         |               |                |                 |       |
| Adult female            | 6/209                   | 0/209         | 71/209         | 78/209          |       |
| Adult male              | 1/155                   | 1/155         | 41/155         | 43/155          |       |
| Nymph                   | 1/219                   | 0/219         | 54/219         | 55/219          |       |
| Larva                   | 0/8                     | 0/8           | 0/8            | 0/8             |       |
| **Amblyomma cajennense**|                         |               |                |                 |       |
| Adult female            | 0/5                     | 0/5           | 1/5            | 1/5             |       |
| Adult male              | 0/6                     | 0/6           | 0/6            | 0/6             |       |
| Nymph                   | 0/19                    | 0/19          | 9/19           | 9/19            |       |
| Larva                   | 0/1                     | 0/1           | 0/1            | 0/1             |       |
| **Amblyomma maculatum** |                         |               |                |                 |       |
| Adult female            | 1/22                    | 0/22          | 2/22           | 3/22            |       |
| Adult male              | 1/29                    | 2/29          | 2/29           | 5/29            |       |
| Nymph                   | 0/6                     | 0/6           | 0/6            | 0/6             |       |
| Larva                   | 0/0                     | 0/0           | 0/0            | 0/0             |       |
| **Dermacentor variabilis** |                        |               |                |                 |       |
| Adult female            | 1/90                    | 0/90          | 2/90           | 3/90            |       |
| Adult male              | 0/63                    | 0/63          | 2/63           | 2/63            |       |
| Nymph                   | 0/4                     | 0/4           | 0/4            | 0/4             |       |
| Larva                   | 0/0                     | 0/0           | 0/0            | 0/0             |       |
| **Dermacentor andersoni** |                      |               |                |                 |       |
| Adult female            | 0/2                     | 0/0           | 2/2            | 2/2             |       |
| Adult male              | 0/0                     | 0/0           | 0/0            | 0/0             |       |
| Nymph                   | 0/0                     | 0/0           | 0/0            | 0/0             |       |
| Larva                   | 0/0                     | 0/0           | 0/0            | 0/0             |       |
| **Ixodes scapularis**   |                         |               |                |                 |       |
| Adult female            | 1/52                    | 0/52          | 42/52          | 43/52           |       |
| Adult male              | 0/1                     | 0/1           | 0/1            | 0/1             |       |
| Nymph                   | 0/6                     | 0/6           | 3/6            | 3/6             |       |
| Larva                   | 0/0                     | 0/0           | 0/0            | 0/0             |       |
| **Total**               | 12/897                  | 4/897         | 231/897        | 247/897         |       |

*Table only includes ticks originating in Texas that tested positive for *Borrelia*, *Ehrlichia*, or *Rickettsia* spp. One hundred thirty-seven *Rhipicephalus sanguineus*, 12 unidentified *Amblyomma* spp., 6 unidentified *Ixodes* spp., 3 *Otoobius megnini*, 2 unidentifiable ticks and 1 specimen each of *Amblyomma imitator*, *Amblyomma inornatum*, *Dermacentor albipictus*, *Dermacentor nigrolineatus*, and *Ixodes woodi* were additionally submitted from Texas.