ers and their family members were advised that they should be tested for HBV infection and receive HBV vaccination if test results were negative. Local health authorities were advised that commercial sex workers and their clients should be vaccinated to prevent HBV infection.

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Ehrlichia Prevalence in Amblyomma americanum, Central Texas

To the Editor: Ehrlichia chaffeensis and E. ewingii, agents of human monocytic ehrlichiosis and ehrlichiosis ewingii, respectively, are transmitted by the lone star tick, Amblyomma americanum, which is found from west-central Texas northward to Iowa, and southeastward to the Atlantic Coast (1). In A. americanum, E. chaffeensis has been found in several states, while E. ewingii has only been found in North Carolina, Florida, and Missouri (1,2). E. ewingii infection in white-tailed deer (Odocoileus virginianus), a potential reservoir, has been found in the states mentioned previously as well as in Kentucky, Georgia, and South Carolina (3,4).

Human ehrlichioses are underdiagnosed in the United States and may be as prevalent as Rocky Mountain spotted fever in some areas (1). Ehrlichioses are prevalent in Texas, and fatal cases have been reported (1,5). This study was conducted to examine ticks from central Texas for Ehrlichia and provide information to increase public health awareness of this problem. Adult A. americanum ticks were collected from a 38.8-hectare game fenced-pasture (Plot #8) in the Kerr Wildlife Management Area, Kerr County, Texas. Ticks were trapped by using blocks (approximately 85 g) of dry ice centered on smooth, white, nylon cloths measuring approximately 1 m². These traps were placed on the ground in the brush or in areas under tree canopies for approximately 1 h.

Trapped adult A. americanum were frozen in liquid nitrogen and then bisected with a sterile scalpel. Halves of the bisected ticks were stored at −80°C. The other halves were pooled in groups of six. DNA was extracted from these pools by using the QIAamp DNA Mini Kit (Qiagen Inc., Valencia, CA), and evaluated by using a nested species-specific 16S rRNA gene polymerase chain reaction (PCR) for E. chaffeensis and E. ewingii, with E. canis as a negative control. The first-round primers were genus-specific for Ehrlichia (ECC and ECB) were separated by electrophoresis. The 478-bp band was recovered using the QIAquick Gel Extraction Kit, then cloned into the pCR2.1-TOPO vector with the TOPO TA cloning kit (BD Biosciences Clontech, Palo Alto, CA). DNA sequences were obtained from both directions of the insert in the recombinant plasmids by using PE Applied Biosystems (Foster City, CA) 373XL automated DNA sequencers in the UTMB Sequencing Core.

Of the 66 adult A. americanum ticks examined, 5 were positive for E. ewingii (7.6%). The 16S rRNA gene sequences from these five positive samples were most similar to the E. ewingii 16S rRNA gene sequence (GenBank accession no.U96436). Sequence variations are summarized in the Table. These mutations may result from polymerase errors prior to cloning. E. ewingii has never been cultured or handled by our laboratory, and all negative controls for the nested PCR were negative, minimizing the possibility of false-positive results.

This is the first report of ticks infected with E. ewingii in states other than North Carolina, Florida, or Missouri. Ticks are found in damp
wooded areas (1,9). Seasonal population changes have been associated with climatic factors, including precipitation, temperature, and day length (9–11). These ticks were collected during August, one of the hottest months of the year in Texas, with temperatures averaging 33° C. Adult ticks are more abundant earlier in the summer, and the actual prevalence of *E. ewingii* infection may be higher. August is a dry month in Texas, with climatic factors, including precipitation, temperature, and day length changes having been associated with the establishment of an epidemiologic agent of human ehrlichiosis. Infection rates of *Dermacentor andersoni* and *Amblyomma americanum* ticks from central Texas, compared to the partial 16S rRNA gene sequence of *Ehrlichia ewingii* in Genbank (accession U96436)*

|                | G16 | A93G | A157G | T190C | A429G | C474 |
|----------------|-----|------|-------|-------|-------|------|
| Tick B5        | –   | –    | –     | +     | –     | –    |
| Tick B7        | +   | –    | –     | +     | –     | –    |
| Tick D1        | –   | +    | +     | –     | –     | +    |
| Tick D2        | –   | +    | +     | –     | –     | –    |
| Tick D4        | –   | –    | –     | –     | –     | +    |

+, mutation present, –, mutation not present.

A single positive-nested PCR reaction should not be considered sufficient for positive identification of the organism. Sequencing of the outer PCR product, or another confirming method, should be used to positively identify the organism. Primers directed to more divergent sequences, such as the dsb gene, should be utilized in place of, or in addition to, 16S rRNA gene PCR (14).

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