DETECTION OF THE AGENTS OF HUMAN EHRlichioSES IN IxOdID TICKS FROM CALIFORNIA

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Abstract. A study was conducted in northern California to estimate the prevalence and distribution in ixodid ticks of the rickettsial agents of human monocytic (HME) and human granulocytic (HGE) ehrlichioses. More than 650 ixodid ticks were collected from 17 sites in six California counties over a 15-month period. Ehrlichia chaffeensis, the causative agent of HME, was detected by a nested polymerase chain reaction (PCR) in Ixodes pacificus (minimum infection rate [MIR] = 13.3%) and Dermacentor variabilis (infection rate = 20.0%) from a municipal park in Santa Cruz County. The HGE agent was detected by nested PCR in I. pacificus adults from a heavily used recreational area in Alameda County (MIR = 4.7%) and a semirural community in Sonoma County (MIR = 6.7%). Evidence of infection with Ehrlichia spp. was not detected in D. occidentalis adults or I. pacificus nymphs. This study represents the first detection of E. chaffeensis in California ticks and the first report of infection in Ixodes spp. The competency of I. pacificus to be coinfect with and to transmit multiple disease agents, including those of human ehrlichioses and Lyme disease, has yet to be determined.

Prior to 1986, human infection with rickettsia of the genus Ehrlichia was not known to occur in the western hemisphere. However, within the last decade, two previously unrecognized forms of human ehrlichiosis have been identified in the United States. Human monocytic ehrlichiosis (HME), caused by E. chaffeensis, and human granulocytic ehrlichiosis (HGE), caused by an as yet unnamed Ehrlichia species, are intracytoplasmic infections of mononuclear and polymorphonuclear leukocytes, respectively. Ehrlichia chaffeensis is closely related to E. canis, a pathogen of canids.1 The HGE agent is very closely related to or synonymous with E. equi2,3 and E. phagocytophila,4 previously recognized pathogens of equids and domestic ruminants.

Human monocytic ehrlichiosis was first recognized in a visitor to Arkansas in 1986,5 and HGE was first reported in 12 patients from Minnesota and Wisconsin in 1994.6 In California, the first cases of HME and HGE were described in 1994 and 1995, respectively.7,8 Although several cases of human ehrlichioses have been reported from the western United States, most HME cases have been reported from the southern states and most HGE cases from the midwestern-northeastern states.9

The agents of HME and HGE are transmitted to humans through the bite of an infected tick. Many HME patients have reported tick exposure or tick bite in the three weeks preceding onset of their illness.10 In the southern United States, E. chaffeensis has been detected in the Lone Star tick Amblyomma americanum L.,12 and the American dog tick Dermacentor variabilis Say;13 whereas in the northeast and midwest, the HGE agent has been detected in the deer tick Ixodes scapularis Say.14,15 White-tailed deer are potential reservoirs for E. chaffeensis in the southern states,16-18 and white-footed mice are reservoirs of the HGE agent in the northeastern states.19 The reservoirs of the etiologic agents of human ehrlichioses in California are unknown.

In California, the western black-legged tick I. pacificus Cooley & Kohls is the probable vector for HGE20 and E. equi,21 whereas the vector for HME has not been described. The objectives of this study were to identify potential vectors of HME and estimate the prevalence and distribution of the agents of HME and HGE in ixodid ticks in northern California.

MATERIALS AND METHODS

Tick collections. Adult and nymphal ticks were collected at 17 sites in six California counties (Table 1) between January 1996 and March 1997. These sites were selected because they are heavily used recreational or residential areas where ticks were known to be abundant. Ticks were collected from vegetation and leaf litter using a standard 1 m2 flannel flag. The species, sex, and number of ticks were recorded at each site, and the ticks were stored alive in the refrigerator until they could be analyzed.

Isolation of DNA and polymerase chain reaction (PCR) amplification. Ticks were analyzed individually or in pools of 2–10 ticks via a nested PCR for evidence of E. chaffeensis or the HGE agent. Ticks were cut in half using a sterile scalpel in 0.5 ml Eppendorf tubes. The DNA from individual or pooled ticks was extracted using the IsoQuick Nucleic Acid Extraction Kit (Orca Research, Bothell, WA) and suspended in 50 µl of sterile water. The extracted DNA was assessed for quality and quantity by PCR amplification of tick mitochondrial DNA using 16S +2 and 16S-1 primers.22

Nest amplification of E. chaffeensis was performed using 16S rRNA primers. The primary reaction used the external set of primers HE1 (5’-CAATTGCTTTAACCCTTTTGGTTAATAAT-3’) and HE20 (5’-GAATTCGCCCATCTCCTTTGGACG-3’). HE1 and HE20 were the primers used in the nested reaction HE1 and HE3 (5’-TATAGGTACCGTCACTATCTTCCCTAT-3’). Nested amplification of the agent of HGE was performed using 16S rRNA primers. The primary primers used in the reaction were GE3a (5’-CACATGCAAGTGAACGGAATTTTCCCTTC-3’) and GE10 (5’-TTCGTTTAAAGAAACCCGGATCTAATC-3’), and nested primers used were GE9 (5’-AACGGATTATCTTTTATAGGCTTGTATC-3’) and GE2 (5’-GCGATTAATTTTAAGAAGCTCCAGG-3’). One microliter of DNA was used in each 20-µl PCR mix-
ture. Each primer was used at a final concentration of 1.0 mol/L. Other reaction components (obtained from Boehringer Mannheim, Indianapolis, IN) were 10 mM Tris, pH 8.3, 50 mM KCl, 1.5 mM MgCl₂, 200 mM of each deoxynucleoside triphosphate, and 1.25 units of Taq polymerase. The PCR amplification for all reactions was performed in a Perkin Elmer (Norwalk, CT) 9600 thermal cycler using the following program: 94°C for 2 min, then 30 cycles at 94°C for 1 min, 50°C for 30 sec, and 70°C for 1 min. A negative control (distilled water) and a positive control (HGE and HME DNA supplied by R. F. Massung, Centers for Disease Control and Prevention, Atlanta, GA) were included with each set of amplifications. Ten microliters of nested PCR products were separated by electrophoresis on a 2.5% wide-range agarose gel (Sigma Chemical Co., St. Louis, MO) stained with ethidium bromide. A reaction was considered positive for *E. chaffeensis* if a 391-basepair (bp) band was visible, and positive for the HGE agent if a 547-bp band was visible.

The PCR results are expressed as a minimum infection rate (MIR) or the minimum percentage of ticks in a pool with detectable ehrlichiae. This calculation is based on the assumption that a PCR-positive pool contains only one positive tick.

**RESULTS**

A total of 664 ticks were collected, including 401 *I. pacificus* adults, 77 *I. pacificus* nymphs, 162 *D. occidentalis* Marx, and 24 *D. variabilis* (Table 1). *Ixodes pacificus* adults were collected from all 17 sites and nymphs were collected from three sites in two counties. *Dermacentor occidentalis* and *D. variabilis* were collected from seven sites in four counties and two sites in two counties, respectively. All *I. pacificus* adults and nymphs were analyzed for the HGE agent and 149 adults and 37 nymphs were tested for *E. chaffeensis*. All *Dermacentor* spp. ticks were analyzed for both the HGE agent and *E. chaffeensis*.

*Ehrlichia chaffeensis* was detected in both *I. pacificus* and *D. variabilis* adults collected from a municipal park located adjacent to the University of California campus in Santa Cruz County (Table 2 and Figure 1). Of the 30 *I. pacificus* tested from this site, *E. chaffeensis* was detected in two pools, one consisting of two females and the other of two males (MIR = 13.3%). Two females and one male of the 15 *D. variabilis* tested were positive (infection rate = 20.0%). Fifteen *D. occidentalis* collected from this park tested negative for *E. chaffeensis*, as did adult and nymphal ticks collected from all other sites. Overall, the MIR of the 149 *I. pacificus* tested from three counties was 3.4% and the infection rate of the 24 *D. variabilis* tested individually from two counties was 12.5%.

The HGE agent was detected in *I. pacificus* adults collected from two counties: Alameda and Sonoma (Table 2 and Figure 1). Of 150 ticks collected from an East Bay Regional Park in Alameda County, four pools of five males each, one pool of 10 females, and two individual males tested positive (MIR = 4.7%). Twenty-seven *I. pacificus* adults collected elsewhere in the county tested negative. Of 45 adult *I. pacificus* collected in a semirural residential community (Community A) in Sonoma County, the HGE agent was detected in one pool of 10 males and two individual females (MIR = 6.7%). The overall MIR of the 401 *I. pacificus* adults tested was 2.0%. The HGE agent was not detected in *I. pacificus* nymphs, *D. occidentalis*, or *D. varia-

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**Table 1**

<table>
<thead>
<tr>
<th>County</th>
<th>No. of sites</th>
<th>No. of <em>Ixodes pacificus</em></th>
<th>No. of <em>Dermacentor variabilis</em></th>
<th>No. of <em>D. occidentalis</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>Alameda</td>
<td>3</td>
<td>177</td>
<td>9</td>
<td>116</td>
</tr>
<tr>
<td>Contra Costa</td>
<td>5</td>
<td>47</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Lake</td>
<td>3</td>
<td>20</td>
<td>0</td>
<td>10</td>
</tr>
<tr>
<td>Mariposa</td>
<td>2</td>
<td>34</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Santa Cruz</td>
<td>2</td>
<td>78</td>
<td>15</td>
<td>15</td>
</tr>
<tr>
<td>Sonoma</td>
<td>2</td>
<td>45</td>
<td>0</td>
<td>21</td>
</tr>
<tr>
<td>Total</td>
<td>17</td>
<td>401</td>
<td>24</td>
<td>162</td>
</tr>
</tbody>
</table>

* Nymphal *I. pacificus* were collected and tested from Lake (n = 40) and Sonoma (n = 37) counties.

**Table 2**

<table>
<thead>
<tr>
<th>County</th>
<th>Site</th>
<th>Ehrlichia chaffeensis</th>
<th>HGE agent <em>I. pacificus</em></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td><em>Ixodes pacificus</em></td>
<td><em>Dermacentor variabilis</em></td>
</tr>
<tr>
<td></td>
<td></td>
<td>No.</td>
<td>MIR (%)</td>
</tr>
<tr>
<td>Alameda</td>
<td>Regional Park</td>
<td>22</td>
<td>0</td>
</tr>
<tr>
<td>Santa Cruz</td>
<td>Municipal Park</td>
<td>30</td>
<td>13.3</td>
</tr>
<tr>
<td>Sonoma</td>
<td>Community A</td>
<td>22</td>
<td>0</td>
</tr>
<tr>
<td>Other</td>
<td></td>
<td>75</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>149</td>
<td>3.4</td>
</tr>
</tbody>
</table>

* Minimum infection rate is calculated based on the assumption that a PCR-positive pool contains only one positive tick.
Adult *I. pacificus* and *D. variabilis* were found to be infected with *E. chaffeensis*, the causative agent of HME. This is the first detection of *E. chaffeensis* in California ticks. In addition, although *E. chaffeensis* has been found in *D. variabilis*\(^1\) and *A. americanum*\(^12\) in the southern United States, this rickettsia has not been reported previously in *Ixodes* spp. The MIR of 3.4% in *I. pacificus* and the infection rate of 12.5% in *D. variabilis* exceed the infection rate of 1.2% found in *A. americanum* adults collected in Missouri and North Carolina.\(^12\) However, the tick infection rates of the present study should be considered preliminary due to the comparatively small number of ticks (149 *I. pacificus* and 24 *D. variabilis*) and sites sampled. Further collection and testing of *I. pacificus* and *D. variabilis* in Santa Cruz County and elsewhere in California will likely modify these estimates to an unknown degree. Both of these species are prevalent and widely distributed in California.\(^24\) Also, although the presence of *E. chaffeensis* was demonstrated in *I. pacificus* and *D. variabilis*, their competency as vectors of *E. chaffeensis* remains undetermined.

This study represents the first report of the HGE agent in *I. pacificus* from heavily populated Alameda County in the San Francisco Bay Area. The East Bay Regional Park is located near the city of Oakland, and its extensive network of trails are used by residents year round, including during the winter months when populations of *I. pacificus* adults typically peak.\(^25\) The 4.0% MIR observed for Alameda County (4.7% for the regional park) exceeds that of any other California county from which ticks have been tested for the agent of HGE.\(^20\) Although no cases of HGE have been detected in residents of Alameda County, the risk of exposure to this disease agent is potentially greater than the risk of acquiring Lyme disease because, according to a 1996 study,\(^26\) less than 1.0% of the *I. pacificus* tested from this region were positive for *Borrelia burgdorferi* Johnson, Shmid, Hyde, Steigerwalt & Brenner.

In Sonoma County, *I. pacificus* were collected from a small semirural community where a seroepidemiology study had been conducted to evaluate the risk of human ehrlichiosis, Lyme disease, and babesiosis.\(^27\) Of the 230 residents participating in the study, 0.4% and 4.6% were seroreactive to *E. equi* and *E. chaffeensis*, respectively. The detection of the HGE agent (which cross-reacts with *E. equi*) in ticks from this community further substantiates that these residents are at risk of contracting HGE. The number of ticks tested from this community was small; a larger sample may yield ticks infected with *E. chaffeensis* in addition to the HGE agent. Evidence of HGE agent infection in *I. pacificus* has been previously reported from a Sonoma County community approximately 80 km to the north of our collection site.\(^20\)

The HGE agent has to date been reported in adult *I. pacificus* collected from six California counties: Alameda, El Dorado, Orange, Santa Cruz, and Sonoma counties\(^20\) and Napa County (Crawford-Miksza L., unpublished data). These counties represent several distinct geographic regions of California, including the Sierra Nevada foothills, the San Francisco Bay area, the north coastal region, and the Los Angeles Basin. Although clinical cases of HGE have been confirmed only from Santa Cruz County,\(^44\) residents and visitors to other regions of the state may be at risk of contracting HGE. The overall MIR of 2.0% for the HGE agent in *I. pacificus* adults in this study is greater than the MIR of 0.8% reported previously,\(^20\) and is comparable to the infection rate of *B. burgdorferi* in *I. pacificus* adults collected throughout California.\(^28\) Studies conducted in northern California have indicated a higher infection rate of *B. burgdorferi* in nymphal *I. pacificus* than in adults,\(^29,30\) and this could be the case for the HGE agent although too few nymphs have been tested to reliably estimate the infection rate of this life stage. Studies conducted elsewhere in the United States report higher HGE agent infection rates in *I. scapularis* than those observed in *I. pacificus* from California. For instance, in Wisconsin 7.9% of 89 *I. scapularis* adults were PCR positive,\(^31\) and in Connecticut, approximately 50% of 118 *I. scapularis* adults tested positive.\(^32\)

The identification of the agents of HME and HGE in *I. pacificus*, in addition to the etiologic agent of Lyme disease, suggests the possibility of coinfection and cotransmission of several disease agents within a given tick. Although more than one pathogen has not been identified in a single tick from California, there is evidence elsewhere in the United States that a single *I. scapularis* can be coinfected with *B. burgdorferi* and the HGE agent,\(^31\) and that patients may suffer simultaneously from more than one tick-borne disease.\(^32-34\) Further studies need to be conducted in California to detect this phenomenon, define the distribution of ticks infected with ehrlichiae, and determine the reservoirs of these disease agents.

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![Diagram of California counties: Alameda, Contra Costa, Marin, Napa, Santa Cruz, San Mateo, San Francisco, Solano, Sonoma, and Sonoma. PCR positive regions are marked by a red square with "E. chaffeensis" and a green square with "HGE agent." PCR negative regions are marked by a black square.](Image)

**Figure 1.** California counties where ixodid ticks were collected for polymerase chain reaction (PCR) analysis. HGE = human granulocytic ehrlichiosis.
Sonoma Mosquito and Vector Control Districts, respectively, for assistance with tick collecting. We thank Robert Massung (Centers for Disease Control and Prevention, Atlanta, GA) for providing our laboratory with positive controls and primer sequences, and Curtis Fritz for critical review of the manuscript. Financial support: This research was supported in part by an appointment to the Postgraduate Internship Program at the U.S. Army Center for Health Promotion and Preventive Medicine (USACHPPM) administered by the Oak Ridge Institute for Science and Education through an interagency agreement between the U.S. Department of Energy and the USACHPPM.

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