Abstract. Dusky-footed wood rats (Neotoma fuscipes Baird) and two species of Peromyscus mice (P. maniculatus Wagner and P. truei Shufeldt) were collected over a 16-month period from three sites in Sonoma County, California. Blood was collected from 93 wood rats and 177 mice and serum or plasma was tested for seroreactivity with Ehrlichia phagocytophila sensu lato (also known as the human granulocytic ehrlichiosis agent). Thirty-five (37.6%) wood rats and 15 (8.5%) mice were seropositive. Positive Neotoma serology by site ranged from 9.4% to 62.1%. Polymerase chain reaction (PCR) testing for the Ehrlichia groEL heat shock operon was performed on all the seropositive and selected seronegative wood rats; 24 (68.6%) seropositive animals were PCR positive. Two seroreversions and no seroconversions were detected among 18 of the seropositive wood rats that were recaptured and tested multiple times (range = 2–6). Fourteen (77.8%) of the 18 were also PCR positive with six of these positive at every testing point (range = 2–6). One wood rat remained serologically and PCR positive in six specimens collected over a 14-month period. One male of 84 questing adult Ixodes pacificus Cosley & Kohls collected was PCR-positive for E. phagocytophila. Borrelia burgdorferi, the agent of Lyme disease, was cultured from ear punch biopsies from six of seven E. phagocytophila seropositive and one of four seronegative wood rats.

INTRODUCTION

Ehrlichiosis is a tick-borne disease caused by obligate intracellular organisms that infect humans and animals. Three causes of human ehrlichiosis are recognized in the United States: Ehrlichia chaffeensis, E. ewingii, and E. phagocytophila sensu lato. Human monocytic ehrlichiosis was first described in 1986, but it was not until 1991 that the causative agent was determined to be E. chaffeensis. Human granulocytic ehrlichiosis (HGE) was first described in 1994 in the upper Midwest. The etiologic agent of this disease has been known for some time as the HGE agent, but subsequent work has shown that it is conspecific with E. phagocytophila and E. equi. In 1999, a different agent of granulocytic ehrlichiosis in humans was discovered when E. ewingii was associated with disease in the southeastern United States.9

The ehrlichiae are transmitted to humans and other mammals through the bites of infected ticks. In the mid-Atlantic, southern, and southeastern states, E. chaffeensis is maintained in a natural cycle involving the lone star tick (Amblyomma americanum L.) and the white-tailed deer (Odocoileus virginianus Zimmermann), which serves as a reservoir. Ehrlichia ewingii may be transmitted by the same tick species, but the reservoir is unknown. In the midwestern and northwestern states, the vector of E. phagocytophila is the deer tick (Ixodes scapularis Say), while the white-footed mouse (Peromyscus leucopus Rafinesque) serves as one reservoir. To date there have been nine confirmed cases of autochthonous human ehrlichiosis in California, three attributed to E. chaffeensis and six attributed to E. phagocytophila. The first case of E. chaffeensis infection detected in California occurred in 1994. The tick vector in California has not yet been confirmed, although I. pacificus Cosley & Kohls and Dermacentor variabilis Marx ticks collected from a site in Santa Cruz County have yielded bands of expected size for E. chaffeensis by the polymerase chain reaction (PCR), with minimum infection rates of 13.3% and 20.0%, respectively. The first case of E. phagocytophila infection in California was diagnosed in 1995. Ixodes pacificus appears to be the vector of granulocytotropic ehrlichiae in California. These nine cases and all equine granulocytic ehrlichiosis cases have been reported only north of Monterey County (California Department of Health Services, unpublished data). The first evidence that E. phagocytophila-like agents may be associated with the genus Neotoma in the western United States was provided by Nicholson and others. Seropositive rodents were identified in California and Colorado. The dusky-footed wood rat (Neotoma fuscipes Baird) was subsequently implicated by serologic and molecular data as a reservoir for the agent of both human and equine granulocytic ehrlichiosis in northern California. Because little is known of the natural history of ehrlichiae in the western United States, or in individual animals, the objectives of this study were to describe the temporal dynamics of serum antibody to E. phagocytophila in wood rats and evaluate its implications for disease transmission.

MATERIALS AND METHODS

Pilot sampling was conducted in Placer, Santa Cruz, and Sonoma counties, California. These counties were chosen because confirmed cases of HGE had been previously identified (Santa Cruz), previous studies had detected granulocytotropic ehrlichiae in ticks and serologic titers to these organisms in human residents (Sonoma), and a Neotoma study was in progress in habitat similar to that in an adjacent county where equine cases of ehrlichiosis had occurred (Placer). Serum samples were collected from 13 rodents in Santa Cruz County, 27 in Sonoma County, and 30 in Placer County. Sera collected from rodents were tested for antibody reactivity to E. phagocytophila antigens by immunofluores-
ence assays. Positive samples were obtained in May 1997 from one of three sites in Sonoma County. No specimens collected from Placer or Santa Cruz Counties were positive. The Sonoma County site was selected for further detailed study.

**Study area (sites) habitat and topography.** The Sonoma study area was located near an area where a previous sero-epidemiologic study had shown that 23% of 230 local residents were seroreactive to antigens from one or more of four tickborne disease agents, including two *Ehrlichia* species. The study area was a partially disturbed combination oak woodland and chaparral habitat (122°32′30″ W, 38°20′35″ N) on the northeast-facing slope of Sonoma Mountain (840–920 feet above sea level). The predominant tree species included live oaks (*Quercus agrifolia* Nee, *Q. wislizenii* Candolle), madrone (*Arbutus menziesii* Pursh), and California bay (*Umbellularia californica* Nuttall). Some apple and plum trees remained from abandoned orchards. Other vegetation included coyote brush (*Baccharis pilularis* Candolle), poison oak (*Rhus diversiloba* Torrey & Gray), soap plant (*Chlorogalum* spp.), ferns, and wild oats (*Avena* spp.). An initial survey was performed to locate wood rat huts and determine the size of the study area and the number of traps and person-hours needed for the study. Because wood rat huts were focally distributed, the study area was subdivided into three surveillance sites. Site A was a chaparral habitat composed predominately of coyote brush, very thick in some areas, interspersed with an occasional abandoned orchard tree. Site B was a mixture of oak woodland (40%) and chaparral (60%). The trap stations in Site B chaparral had more canopy cover than the more open site A. Site C was composed of approximately a 50% mixture of oaks, madrone, and California bay trees with a poison oak understory, and 50% open oak-grassland combination.

**Mammal trapping and processing.** Rodent traps were set out over two consecutive nights each month in July, August, September, and October 1997 and in May (1 night), June, August, and October 1998. Due to inclement weather, trapping was not conducted from late fall to early spring. Traps were set every night from 3:30 PM to 5:00 PM and picked up the following morning between 8:00 AM and 9:30 AM. Rodents were collected in National (Tomahawk) traps (TomahawkLive Trap Co., Tomahawk, WI) (41 × 14 × 14 cm) baited with peanut butter and oats or in Sherman traps (H. B. Sherman, Tallahassee, FL) (25 × 3 × 3 cm) baited only with oats. Ten trapping stations were established for sites A and C and 15 stations for site B. Where possible, stations were positioned near wood rat huts. A lathe stake and flagging material were placed at each station and sequentially numbered. Each station included two National and two Sherman traps. The rodents were collected, transported, and processed in accordance with published guidelines.

The rodents were anesthetized with ethyl ether and ectoparasites were removed by combing. Fleas were placed in either 2% saline or 70% ethanol for later identification. Rodents were identified to species, gender, and reproductive status. A total head and body measurement was recorded. Blood was collected by cardiocentesis and placed in 3-ml Vacutainer tubes (Becton Dickinson, Rutherford, NJ) containing EDTA (K3) as an anticoagulant. All blood samples were coded and shipped to the Centers for Disease Control and Prevention for testing. Attached nymphal and adult ticks were removed by forceps and preserved in 70% ethanol for later identification and testing on all trapping dates. Larval ticks were removed only on the July and August 1997 dates; after these dates larvae were taken only inadvertently when mistaken for nymphs. *Neotoma* were marked with uniquely numbered ear tags (National Band & Tag Co., Newport, KY) and returned to their respective traps to recover prior to release at their capture site.

**Serologic testing.** An indirect immunofluorescence assay (IFA) for detecting rodent immunoglobulins reactive with *E. phagocytophila* was performed as previously described. Positive and negative control sera were included in all assays. Geometric mean titers (GMTs) were calculated for each of the May–June, July–August, and September–October trapping periods.

**Extraction of DNA and PCR assay.** The DNA was extracted from whole-blood specimens (50 μl), blood clots, and individual ticks. All seropositive wood rats, most seropositive mice, and a subset of seronegative specimens were tested by PCR assays with primers directed against the *groESL* heat shock operon of *Ehrlichia* spp. as described by Nicholson and others.

**Isolation of spirochetes.** One or two 2-mm ear punch biopsy samples were collected from all wood rats trapped in May 1998. The samples were placed in a 13 × 100 mm screw-capped test tube with 7 ml of Barbour, Stoenner, Kelley (BSK) II medium containing rabbit serum, rifampin (50 μg/ml), phosphomycin (100 μg/ml), and amphotericin B (10 μg/ml). Aliquots of other *Borrelia burgdorferi* Johnson, Schmid, Hyde, Steigerwalt & Brenner cultures were inoculated into BSK II medium as positive culture controls. Cultures were maintained at 31–33°C in a CO2-enhanced environment. Wet mounts were examined approximately weekly by dark-field microscopy for the presence of spirochetes. All wet mount-positive cultures were confirmed as *B. burgdorferi* by IFA. Further analysis of the cultured spirochetes was not performed.

**Tick flagging samples.** Adult and nymphal ticks were collected in and around the three trapping sites by flagging vegetation and leaf litter with a 1-m2 Bannel cloth. Sampling was performed on six dates between June 11, 1997 and January 14, 1999. The number of people flagging varied between two and five. The flagging time varied from 90 to 150 min. The species, sex, life stage, and number of ticks were recorded for each collection. Ticks were stored in 70% ethanol and identified to species using morphologic keys from Furman and Loomis.

**Statistical methods.** Chi square 2 × 2 contingency tables were used to analyze wood rat serology data by sites. In all tests a 5% level of probability was established for rejection of the null hypothesis. Data were grouped in the following monthly pairs for seasonal analysis: May and June, July and August, and September and October.

**RESULTS**

A total of 1,946 trap-nights were compiled over the 16-month study period; 386 captures of six rodent species were tallied for an overall trap success rate of 19.8%. Rodents collected were 93 wood rats (*N. fuscipes*, with 101 recap-
from 18 of the IFA-positive wood rats (range three-fold increase in mean titers from the May–June to the for September–October (Figure 1). There was a two- to for the May–June period, 567.4 for July–August, and 591.1 from 16 to 8,192 with a GMT of 362. The GMTs were 97 and site C (\(C\)) significantly less than the proportion at site B (\(B\)). The percentage of individual wood rats serologically positive varied by site as follows: Site A \(A\) was 36.2% (Table 1). The proportion of wood rats seropositive for \(E.\ phagocytophila\) were found in 35 (37.6%) of the wood rats tested from site C were culture positive for \(B.\ burgdorferi\) trapped in May 1998. Two wood rats (# 2392 and 2398, Table 2) maintained positive titers in all six samples collected over a 14-month period.

Fifteen mice were antibody positive for the HGE agent: 10 (11.1%) of 90 \(P.\ maniculatus\) and five (5.7%) of 87 \(P.\ truei\). The GMT for \(P.\ maniculatus\) was 45, with an endpoint titer range of 16–1,024. Positive \(P.\ maniculatus\) were present at all three sites. The GMT for \(P.\ truei\) was 147, with an endpoint titer range of 32–1,024. All positive \(P.\ truei\) were from site C. Five specimens of \(R.\ megalotis\) and one \(M.\ californicus\) were tested and found to be seronegative. No testing was performed on the four \(Sorex\) spp. caught.

Amplification of DNA by the PCR was performed on samples from all serologically positive wood rats. At least one specimen from 24 (68.6%) of the 35 seropositive wood rats was positive by the PCR. Fourteen (77.8%) of 18 multi-captured and 10 (58.8%) of 17 single capture wood rats were PCR positive (Table 2). PCR-positive specimens were collected at every sampling period (range = 2–7). Six rodents were consistently PCR positive each time they were sampled; one rodent was PCR positive six times over a 14-month period. The 11 wood rats with multiple PCR-positive results averaged 3.1 consecutive positive samplings over an average of 5.1 months of resampling. The PCR testing was also performed on 11 serologically positive mice. One of three \(P.\ truei\) was PCR positive while all eight \(P.\ maniculatus\) tested were negative.

Ear punch biopsies were obtained from all 11 wood rats trapped in May 1998. \(Borrelia\ burgdorferi\) were cultured from seven (63.6%) of these. Six of these seven were also serologically positive for \(E.\ phagocytophila\). All five wood rats tested from site C were culture positive for \(B.\ burgdor-

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**Table 1**: Prevalence (%) of serologic antibodies to granulocytotropic ehrlichiae in *Neotoma fuscipes* by sex and capture site, Sonoma County, California, 1997–1998

<table>
<thead>
<tr>
<th>Site</th>
<th>Male</th>
<th>Female</th>
<th>Total</th>
<th>Total samples positive(^*)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>3/13 (23.1)</td>
<td>0/19 (0)</td>
<td>3/32 (9.4)</td>
<td>4/67 (5.9)</td>
</tr>
<tr>
<td>B</td>
<td>6/17 (35.3)</td>
<td>8/15 (53.3)</td>
<td>14/32 (43.8)</td>
<td>30/68 (44.1)</td>
</tr>
<tr>
<td>C</td>
<td>10/14 (71.4)</td>
<td>8/15 (53.3)</td>
<td>18/29 (62.1)</td>
<td>42/59 (71.2)</td>
</tr>
<tr>
<td>Total</td>
<td>19/44 (43.2)</td>
<td>16/49 (32.7)</td>
<td>35/93 (37.6)</td>
<td>76/194 (39.2)</td>
</tr>
</tbody>
</table>

\(^*\) This figure reflects total samples tested from animals including multiple sera from individual wood rats caught at several sampling periods.

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**Figure 1**: Geometric mean titers (with 95% confidence limits) by season in seropositive *Neotoma fuscipes* in Sonoma County, California, 1997–1998.
feri, as well as seroreactive to E. phagocytophila. Four of these five animals were also PCR positive for ehrlichiae giving a coinfection rate of 80%. The one wood rat (#2390) not PCR positive for granulocytic ehrlichiae had been seropositive and PCR positive on the three previous collections. The one wood rat (#2390) not PCR positive for granulocytic ehrlichiae had been seropositive and PCR positive on the three previous collections.

A total of 734 ticks were collected by flagging, including 238 I. pacificus adults, 400 I. pacificus nymphs, 14 D. occidentalis adults, four D. occidentalis nymphs, and 78 Hae-maphysalis leporisplalu-stris Packard nymphs (Table 3). Tick collection rates ranged from 0.2/person-hr for adults of Ixodes in May 1998 and for adults of Dermacentor in June 1997 to 21.1/person-hr for I. pacificus nymphs in May 1998 (Table 3). Questing tick populations at the trapping sites were compared on two flagging dates. The percentages of total I. pacificus ticks collected (n = 142) by site were 6% for site A, 44% for site B, and 50% for site C.

Rodent tick burdens were very light (Table 4). Of 172 wood rats examined only three adult ixodid ticks were found. Two females and one male I. spinipalpis Hadwen and Nuttall (= I. neotomae Cooley) were collected from two wood rats at site C; both rodent hosts tested positive for HGE serologically and by PCR. Four nymphs each of I. woodi Bishop and I. pacificus were found on six wood rats. No Ixodes spp. nymphs were found on the mice. Forty-four D. occidentalis nymphs were found on 33 wood rats and seven on seven Peromyscus mice. Seventeen additional nymphs were removed from the wood rats during the July 1997 collection but were not identified to species.

PCR testing was performed on 10 D. occidentalis nymphs that were removed from the wood rats. Eighty-four adult and 34 nymphal I. pacificus, along with nine adult D. occidentalis, flagged in the area of the wood rat huts and trapping stations were also tested by PCR. Only one male I. pacificus (1.2%) was PCR positive.

Fleas were recovered from rodents during six of eight trapping periods. Flea indices for wood rats were between 1.4 (range = 0–6) in June 1998 and 7.4 (range = 0–46) in October 1998. Of 518 fleas collected, 513 were identified to species (Table 4). Five species of fleas were obtained from wood rats with 457 (96.8%) being Orchopeas sexdentatus Jordan. Two fleas of this species were found on the mice. Five species of fleas were recovered from the mice with 34 (82.9%) of 41 being Opisodasys keeni (Baker). Two fleas of this species were also recovered from wood rats.

**DISCUSSION**

This study provides strong evidence that ehrlichiae of potential human disease significance circulate in the bloodstream of dusky-footed wood rats in northern California over a prolonged period. This is the first reported evidence of N. fuscipes coinfected with E. phagocytophila and B. burgdor-
**Table 4**

Flea and tick ectoparasites of wood rats and mice by capture date, Sonoma County, California, 1997–1998

<table>
<thead>
<tr>
<th>Host</th>
<th>Ectoparasite</th>
<th>Jul</th>
<th>Aug</th>
<th>Sep†</th>
<th>Oct†</th>
<th>May‡</th>
<th>Jun‡</th>
<th>Aug</th>
<th>Oct</th>
<th>Total</th>
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<td>Ticks</td>
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<tr>
<td><em>Ixodes spinipalpus</em></td>
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<tr>
<td><em>I. pacificus</em></td>
<td>(nymphs)</td>
<td>*</td>
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<td>0</td>
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<td>(nymphs)</td>
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<td>0</td>
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* 17 nymphs were submitted to the laboratory before identification was made.
† For September 1997 or October 1998 fleas were not identified.
‡ For May and June 1998 fleas for both species of *Peromyscus* were combined.

Wild caught wood rats have previously been shown to retain infections with *B. burgdorferi* for at least 13–15 months of captivity and may retain borreliosis for life. In this study, wood rats were seroreactive and PCR positive for *E. phagocytophila* for intervals up to 14 months (Table 2). Two seroconversions during the study were noted, but no seroreversions (going from positive to negative). If wood rats are capable of maintaining an infection for a year or more, they are capable of being infective during the periods of peak abundance of *Ixodes* ticks or other potential vectors. Wood rats may remain infected with *E. phagocytophila* for life, or they may be frequently reinfected by nymphal and, to a lesser degree, adult stage *Ixodes* spp.

Humans infected with *E. phagocytophila* do not appear to mount life-long immunity and are susceptible to reinfection. Following initial infection, patients with human ehrlichiosis develop a detectable immune response that peaks approximately one month after acute illness and decreases three months later. A subsequent exposure may precipitate an anamnestic increase in titer from 80 to 2,560 at two and eight days after exposure. A similar reinfection pattern may occur in the wood rat. During the spring to early summer, when nymphal *Ixodes* are most abundant, the wood rat GMT increased two- to three-fold (Figure 1). The four *I. pacificus* nymphs removed from the wood rats were taken between May and August. The transfer of *E. phagocytophila* from infected nymphs to naïve or previously infected wood rats during this time period might explain the observed increase in GMT.

Neotoma infection rates differed significantly among sites. Seroprevalence among wood rats was highest at sites where *I. pacificus* comprised the largest proportion of the tick species present. Site C, the least chaparral-like of the three sites, had the highest percentage of seropositive wood rats (62.1%) as well as the highest number of questing *I. pacificus* (50% of total flagged). In contrast, site A, the chaparral site, had a seroprevalence of 9.4% and contributed only 6% of the *I. pacificus* flagged. This evidence suggests that *I. pacificus* is involved and may be an important contributor in an *E. phagocytophila* maintenance cycle with the wood rat.

*Ehrlichia phagocytophila* was detected by PCR assay in only one *I. pacificus* tick. Although low numbers of questing *I. pacificus* have been found to be infected, others concluded that *I. pacificus* was the most likely vector of *E. equi* in California based on the distribution and life cycle of *I. pacificus* in relationship to spatial and temporal cases of equine granulocytic ehrlichiosis. Equine cases occurred only from Monterey County north, the region of California in which *I. pacificus* is most abundant. The nine human cases of ehrlichiosis acquired in California are even more restricted, occurring along the coast from Santa Cruz County in central California to southern Humboldt County in the north, a distance of approximately 250 miles. This area is prime *I. pacificus* habitat and most Lyme disease cases in California occur in this area. Subadult *I. pacificus* are not a common ectoparasite of wood rats and *I. spinipalpus (= *I. neotoma*) serves as a maintenance vector for *B. burgdorferi*. Nevertheless, larval and nymphal *I. pacificus* ticks do feed at levels on wood rats sufficient to become infected with *B. burgdorferi* as nymphs and adults, allowing transmission to humans to take place. In this transmission cycle, *I. pacificus* is a bridge vector from reservoir wood
rivia to humans and domestic animals. It appears that I. pacificus may serve in the same capacity in the E. phagocytophila cycle in the western United States. In Colorado, Mexican wood rats (N. mexicana Baird) have been found to be co-infected with E. phagocytophila and B. burgdorferi and this enzootic cycle appears to be maintained by I. spinipalpis.34

Fleas were commonly found on the rodents in this study. The range of flea indices is similar to studies conducted in central California over the same study months (Davis RM, unpublished data).35 The high percentage of O. sexdentatus parasitizing the wood rats agrees with studies conducted in central and northern California where this species was the most abundant flea present, representing between 80.7% and 98.3% of total fleas.35,36 In other northern California studies, Opisodays keeni has been identified as the most common flea on P. maniculatus ranging from 33.4% to 72.9% of fleas on the host.37,38

The risk of acquiring E. phagocytophila by humans is still difficult to evaluate. The number of subclinical and undiagnosed cases is unknown. Infection rates in adult I. pacificus in the same study county have ranged from 0.3% to 6.7% with a rate of 2.1% in the nymphal stage.17,19 This is similar to the infection rate for B. burgdorferi in a county that ranked seventh, of 58 California counties, in reported Lyme disease incidence from 1991 to 1999 (California Department of Health Services, unpublished data). It is likely that some E. phagocytophila transmission is taking place as well.

Further studies should be aimed at testing I. pacificus and I. spinipalpus nymphs in northern California and to determine the vector competence of I. spinipalpus for E. phagocytophila. Investigations of the competency of I. pacificus to become co-infected and co-transmit E. phagocytophila and B. burgdorferi would provide valuable data.

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