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Prevalence and distribution of tick-borne pathogens in Santa Cruz County

Kevin Holden
San Jose State University

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**PREVALENCE AND DISTRIBUTION OF TICK-BORNE PATHOGENS IN
SANTA CRUZ COUNTY**

**A Thesis
Presented to
The Faculty of the Department of Biological Sciences
San Jose State University**

**In Partial Fulfillment
Of the Requirements for the Degree
Master Of Science**

**By
Kevin Holden
August 2002**

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APPROVED FOR THE DEPARTMENT OF BIOLOGICAL SCIENCES

Dr. John Boothby

Dr. Sulekha Anand

Dr. Sharron Penn, Amersham Biosciences

APPROVED FOR THE UNIVERSITY

ABSTRACT

PREVALENCE AND DISTRIBUTION OF TICK-BORNE PATHOGENS IN SANTA CRUZ COUNTY

By Kevin Holden

A study was conducted in Santa Cruz County parklands to estimate prevalence and distribution of the agents of Lyme disease, human granulocytic (HGE) and human monocytic (HME) ehrlichiosis in ticks. *Borrelia burgdorferi*, the agent of Lyme disease, was detected by a polymerase chain reaction (PCR) in 44 of 776 (5.67%) *Ixodes pacificus* ticks and in 3 of 58 (5.17%) *Dermacentor variabilis* ticks. The HGE agent was detected in 48 (6.19%) *I. pacificus* ticks and 5 (8.62%) *D. variabilis* ticks. *Ehrlichia chaffeensis*, the agent of HME, was detected in just 5 (0.64%) *I. pacificus* ticks and 4 (6.9%) *D. variabilis* ticks. This study represents the first survey of Lyme and the ehrlichial diseases across multiple areas of Santa Cruz County, and suggests prevalence of *B. burgdorferi* may be higher there than other areas of the state.

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CHAPTER I

Introduction

Tick-borne pathogens are bacterial, viral, or protozoal agents capable of transmission from one host to another via the bite of a tick. These pathogens are found throughout the world and represent a threat to both livestock and human health. Many are considered emerging zoonoses, newly described diseases for which little is known regarding modes of transmission, pathogenesis, and geographic distribution. As incidence of these diseases becomes known, public education on the presence of the infective agents and their arthropod vectors is essential. This study provides prevalence and distribution estimates of several human tick-borne pathogens among adult ticks populations from public use areas in Santa Cruz County, California.

This thesis consists of three chapters and an appendix. Chapter I is a foreword to the research topic, a guide to the contents and organization of the thesis, and a general introduction to Lyme disease and human ehrlichiosis. Chapter II is a report of the work to be submitted for journal peer review. Chapter III provides some additional methodology and an expanded analysis and discussion. An appendix provides data on sampling descriptions and results.

Lyme Disease

Lyme borreliosis, or Lyme disease, is the most frequent human tick-borne disease in the world (Steere 1989). It is a multiphased, multisystem disease characterized by chronic dermatological, musculoskeletal and neurological manifestations. Transmission of the disease is carried out by the bite of an ixodid (hard) tick infected with the spirochete bacterium, *Borrelia burgdorferi* sensu lato. Lyme disease has been described on 6 continents, in at least 20 countries, and in 46 states of the United States of America. In the U.S., it accounts for 95% of all reported tick-borne diseases (Centers for Disease Control and Prevention, Atlanta, GA), with three primary endemic regions: the Mid-Atlantic and New England states, the upper mid-west and the Pacific coast.

A relatively recently described disease, Lyme disease was first recognized in Old Lyme, Connecticut in 1975, where a focal epidemic of skin rashes and subsequent childhood arthritis occurred. Many symptoms of the disease, such as the hallmark erythema migrans rash and ensuing meningital disorders, were previously documented in Europe as early as the end of the 19th century (Barbour and Hayes 1986). However, until the link had been made between this disorder, a tick vector and a bacterial agent, the disease escaped definition. It was determined by the mid 1970s that the cause of the pathology was a bite from an ixodid tick of the genus *Ixodes*. In 1981 a spirochetal bacterium was isolated from the midgut of an *Ixodes scapularis* (black-legged tick; formerly *I. dammini*) tick in New York (Burgdorfer et al. 1982). This organism was subsequently named *B. burgdorferi* after its discoverer, William Burgdorfer, and later recovered from patients with Lyme disease. Since then, several additional species of

ixodid ticks from five genera (*Amblyomma*, *Dermacentor*, *Haemaphysalis*, *Ixodes*, *Rhipicephalus*) have been found to carry *B. burgdorferi* (Dennis et al. 1998). However, the principal vector species are *I. scapularis* in the northeastern and upper midwestern United States, *I. pacificus* (Western Black-legged tick) in the far-western United States, and *I. ricinus* (the Sheep tick) and *I. persulcatus* (the Taiga tick) in Europe and Asia respectively (Sparango et al. 1999). Reservoirs for the disease typically include deer and mice, but may also include birds and lizards in many areas of the world.

The pathogenesis of Lyme disease is complex and is still not completely understood. The etiological agent is the corkscrew-shaped bacterium, *B. burgdorferi* sensu lato, and other closely related species in Europe and Asia. *Borrelia burgdorferi* is a gram-negative spirochete with 7 to 20 periplasmic flagella. It is microaerophilic and is best cultured in BSK II medium at 33°C (Barbour 1984). Despite the gram-negative cell wall, the bacterium does not stain well and is best visualized in the laboratory using darkfield microscopy. Following the bite of a tick infected with *B. burgdorferi*, an erythema migrans "bull's-eye" lesion typically occurs at the site of the bite. The rash spreads uniformly in a circular pattern, covering an area from 5-50 cm, representing migration of the bacterium through the surrounding tissues (Barbour and Hayes 1986). Despite this mild inflammatory response, borreliae are not strongly immunogenic and overall immune responses to infection are limited, possibly due to intracellular localization of the spirochete or other protective mechanisms (Barbour and Hayes 1986). *Borrelia burgdorferi* are capable of invading and infecting a wide range of tissues through mechanisms still being elucidated. Most symptoms of the disease are a direct

result of the body's inflammatory responses to the spirochete antigens. Although antibody is produced, it is not effective in clearing the organism before it localizes into tissues (Barbour and Hayes 1986). Like other species of borreliae, *B. burgdorferi* are believed to be capable of antigenic shift and effectively evade the immune system (Barbour and Hayes 1986). Many of the surface proteins of *B. burgdorferi* are encoded for genes contained in unique linear plasmids, and have a high degree of recombination (Barbour and Restrepo 2000). Interestingly, vaccination against the spirochete using recombinant surface antigens produces a highly effective antibody response on subsequent exposure.

Three clinical stages are classically assigned to Lyme disease, although stages are not exactly defined and may overlap or occur alone. Clinical definition is complicated by the extent of multisystem involvement and mimicry of other diseases. The first stage is characterized by the expanding erythema migrans (bull's eye rash) originating at the site of the tick bite, present in approximately 60-75% of all cases and considered diagnostic for Lyme disease (Ley et al. 1994). Many Lyme disease patients do not recall a tick bite, which is painless. Many non-specific symptoms, which may take several days to weeks to manifest and include headache, lymphadenopathy, fever and other flu-like maladies, earn Lyme disease the nickname "the great imitator" (Steere 1989). These generalized symptoms may be disregarded as a mild cold and appear to resolve without treatment, but may lead to the second stage of the disease, typically within two to three weeks as the spirochete spreads to other sites in the body. During the second stage, *B. burgdorferi* disseminates through the bloodstream and cerebrospinal fluid, causing a systemic

infection (Steere 1989). Second stage Lyme disease symptoms include early-onset arthritis and joint pain, central nervous system involvement (meningitis, dizziness, ocular invasion, paralysis), heart palpitations, and liver involvement. Early diagnosis of the disease by this point is vital to successful treatment preventing progression to chronic stages. Antibiotic treatment (typically with doxycycline or amoxicillin) is usually highly effective in eradicating infection during the first and early second stages.

Stage three is characterized by the development of permanent, chronic sequela, seen in approximately 80% of untreated cases (Steere 1989). These symptoms include chronic arthritis, arthralgias, neurological abnormalities, meningitis, encephalitis, peripheral nerve damage, facial palsy, heart block, congestive heart failure and myopericarditis, lasting as little as a few weeks or many years. Chronic arthritic conditions are believed to result from dissemination of the spirochete into the neural tissues of the joints. Essentially sequestered from the immune system, the spirochete may persist and reactivate later. It is believed that spirochete outer surface protein A (ospA) is primarily responsible for the inflammatory reactions seen in the neural-muscular joint tissue leading to chronic arthritic conditions (Brandt et al. 1990). Such circumstances render antibiotic therapy difficult and in many cases weakly effective, usually requiring much longer treatment than for early stages. Relapses are common and may require the continuous use of therapy. Although Lyme disease is not considered a fatal disease, it can be a severely debilitating one.

Clinical laboratory diagnosis of Lyme disease is dependent on the stage of the disease. A patient presenting a characteristic erythema migrans rash and recent history of

tick exposure should be treated regardless of seropositivity. In some cases, the physician may have the tick tested for the presence of spirochetes or may wish to wait for a serological test, but quite often antibiotics are prescribed at the urging of the patient (Montiel et al. 2002). In cases where secondary or tertiary stage symptoms are observed and Lyme disease is suspected, serological tests are usually performed that include a Western blot. In this case a positive results is based on the detection of patient antibodies to the flagellar antigen of *B. burgdorferi*. Although it is considered the "gold standard" of Lyme disease testing, its sensitivity is limited (59-66%), cross-reactivity with other peptides is possible, and there is currently no standardization of testing protocols for clinical pathologists (Fritz and Vugia 2001). Another disadvantage of serological testing is that previous exposure via vaccination or infection will yield a false-positive result. Positive culture of the organism from erythema migrans lesions is not considered a sensitive method of diagnosis and is difficult due to the complex growth requirements of the bacterium. Testing of patient serum for the presence of unique *B. burgdorferi* genes and detection of spirochetes within ticks is best achieved using newer molecular techniques, particularly amplification of specific markers using polymerase chain reaction (Montiel et al. 2002).

Lyme disease is best prevented by avoiding tick habitats. In tick exposure situations, protective clothing should be worn in order to minimize potential contact including tucking of socks into long trousers and the usage of insect repellents. Control of reservoir mammals in limiting the spread of the disease is largely unpractical (Monsen et al. 1999). Two types of vaccine have been developed: one is an inactivated whole cell

vaccine used in pets and the other, available for human use, is a recombinant vaccine against the OspA antigen. Human vaccines are given to those at high risk, such as park rangers and those working in typical tick habitats. Controversy currently surrounds the current human vaccine as some vaccinated patients who have received it have apparently developed late stage Lyme disease symptoms, such as arthritis (Rose et al. 2001). The recombinant ospA protein used in the vaccine may cause the Lyme disease-like arthritis syndrome by causing an inflammatory reaction in neuro-muscular tissues (Rose et al. 2001).

Tick biology is important in understanding Lyme disease since ticks transmit the spirochete to humans during a blood meal. When ticks are infected, the bacterium resides primarily in the midgut of the tick, but may also disseminate to other tissues, including the ovaries of infected females (Barbour and Hayes 1986). During feeding, *B. burgdorferi* may be transmitted to the host via the salivary glands of the tick. Before inserting their barbed feeding appendage, ticks bring saliva from their midguts in order to facilitate entry through the host dermis and to provide protection against host flora and defense complexes (Barbour and Hayes 1986). The warmth of the incoming blood during feeding activates spirochetes residing in the midgut, which migrate to the salivary glands and are inoculated into the host.

The life cycle of *Ixodes* ticks is complex and typically takes two to three years to complete. Adult ticks mate on large mammals, most often deer, in the late fall or winter. After mating, adults fall off the host where the female lays eggs, which lay dormant until the following summer. Upon hatching, larvae seek a blood meal, commonly feeding on

small mammals such as mice or small birds. Although it has been shown that adult female ticks with disseminated ovarian *B. burgdorferi* infections can lay eggs containing spirochetes (Lane and Burgdorfer 1987), it is believed that during this initial meal larval ticks most commonly become infected (Clover and Lane, 1995). After feeding for approximately 72 hours, ticks drop off and hibernate for the winter on the ground, typically in leaf litter. The following spring the larvae grow and molt into nymphs, which typically feed on larger hosts. Again, the blood meal typically lasts 72 hours, before the engorged nymphal tick falls off to remain inactive until it molts into the adult form the following fall. The adults mate and feed on a wide range of hosts from deer to humans, and may either become infected (if previously uninfected) or continue to serve as vectors.

Since the first clinical cases were reported in 1975, Lyme disease has become the most frequently reported vector-borne disease in the United States (Steere 1989). The incidence of the disease has risen dramatically from 497 cases nationally in 1982 to 16,273 cases reported to the Centers for Disease Control in 1999 (CDC). Since 1982, over 145,000 cases have been reported (CDC), and there is also thought to be a considerable degree of underreporting. Such figures point to better recognition and diagnosis of the disease and possibly increased tick exposure. An increasing trend in urbanization of the human population into previously rural areas where deer and ticks reside may also contribute (Clover and Lane 1995). Furthermore, populations of important reservoirs for Lyme disease, such as deer, are increasing in many areas of the United States possibly due to either a decline in hunting or predation (Dennis et al. 1998).

Consequently, tick vector populations are also believed to be on the rise. Several areas of the United States appear to be endemic for the disease: the New England states, particularly Connecticut, New Jersey and New York; two midwestern states, Wisconsin and Minnesota; and the coastal counties of the Pacific, specifically of northern California where tick vector and reservoir host populations are large, and human urbanization is extensive.

In California, the primary vector of *B. burgdorferi* is the Western black-legged tick, *I. pacificus*, which has been identified in 53 of 58 counties at elevations ranging from sea level to 2,150 meters (Furman and Loomis 1984). These ticks are most easily identified by a distinct reddish-tint to their scapitulum (Furman and Loomis 1984), noticeably present in females. Although other tick species are known to harbor the bacterium, most noticeably *Dermacentor variabilis* (the principal vector in the transmission of Rickettsial typhus, Ehrlichiosis and Q-fever), recent work has cast doubt on their ability to act as competent vectors for Lyme disease (Johns et al. 1998). All life stages of the tick (larval, nymph and adult) are capable of transmitting the disease. Interestingly, the incidence of the disease is much lower in California than other areas of the United States, with only 5% of all documented cases stemming from the west.

The lower incidence of Lyme disease observed in the western United States may be due to factors associated with the feeding habits of nymphal vector ticks. Natural reservoir hosts for *I. pacificus* typically include the white-footed mouse for larvae and nymphs, and deer for adults. Many other animals may serve as suitable hosts, and *I. pacificus* ticks may feed on as many as 31 different species of mammals, 49 species of

birds, and also on lizards (Lane and Quistad 1998). One host, the Western fence lizard (*Sceloporus occidentalis*), may play a role in limiting the spread of the bacterium through animal populations in California. Infected ticks feeding on this lizard apparently lose infectivity due to the presence of an uncharacterized bactericidal factor within the lizard blood (Lane and Quistad 1998). This factor may be a large protein complex within lizard hemolymph that is passed back into the feeding tick, resulting in reduced spirochete levels in the tick and lower prevalence rates in California where lizard populations are relatively large (Lane and Quistad 1998). Current prevalence estimates in California show lower percentages of *I. pacificus* ticks infected with *B. burgdorferi* (approximately 1-2%) than the eastern United States where *I. scapularis* has at least a 50% infectivity rate (Lane and Quistad 1998).

Currently, 100-200 cases of Lyme disease are reported yearly in California, with 1619 cases reported from 1990 to 1999 (CDC). As a result, several northwestern counties of the state have been designated by the CDC as endemic, including Lake and Mendocino counties (CDC). Several studies have attempted to assess public risk by testing tick populations for infection with the spirochete using microscopic methods for identification rather than more specific, sensitive molecular techniques (Sparagano et al. 1999). These studies estimate state infection rates at approximately 1-2% in adult ticks (Lane et al. 1991, Kramer and Beesley 1993), but limited prevalence information exists for many of the 41 counties in California known to harbor *B. burgdorferi* positive ticks (California Department of Health Services). Santa Cruz County, where cases of tick-borne diseases have recently been reported, has very similar environmental conditions

favoring *I. pacificus* and deer habitats to the endemic northwestern counties (Ley et al. 1994). From a public health standpoint, Lyme disease represents a serious threat to those working in tick-infested environments and to the general public in their use of public recreational areas. Despite recent studies implicating the nymphal stages of this tick as having the primary role of transmitting the disease to humans, greater than 40% of reported cases with erythema migrans in California (1991-1999) were acquired during the winter and spring months, when nymphal stages are not thought to be highly active (Monsen et al. 1999). In Santa Cruz County visitation of public parklands occurs year-round due to a persistently mild climate; hence it is important to determine the prevalence and distribution of tick-borne pathogens within recreational use areas of Santa Cruz County.

Human Ehrlichiosis

Rickettsia of the genus *Ehrlichia* are obligate intracellular bacteria and act as important tick-borne pathogens in animals, particularly domestic ruminants (Aleksheev et al 2001). Infection with members of the genus *Ehrlichia* (ehrlichiosis) was first described in 1935 in the United States as a canine disease caused by *Ehrlichia canis* (Baumgarten et al. 1999). Over the next several decades, ehrlichial pathogens were recognized in cattle, goats and horses (*Ehrlichia equi*), and other ruminants (Sparango et al. 1999). In 1953 in Japan, *Ehrlichia sennetsu* was identified as the first ehrlichial human pathogen (Baumgarten et al. 1999). This disease resembles mononucleosis, commonly known as Sennetsu fever, and occurs mostly in Japan. Recently, two

previously unknown species of *Ehrlichia* have emerged as human pathogens in the United States (McQuiston et al. 1999). Human monocytic ehrlichiosis (HME), caused by *Ehrlichia chaffeensis*, was first recognized in Arkansas in 1986 and infects mononuclear leukocytes (Maeda et al. 1987). Human granulocytic ehrlichiosis (HGE), first recognized in Minnesota and Wisconsin in 1994, is caused by an uncharacterized *Ehrlichia* species known as the HGE agent (thought to be *Ehrlichia phagocytophila* or *Ehrlichia equi*), that infects polymorphonuclear leukocytes (Bakken et al. 1994). *Ehrlichia ewingii* was also described as a human pathogen in 1999, with several cases reported from the southeastern United States. Relatively little is known about the life cycles, disease pathogenesis, incidence, and associated vectors and reservoirs of these bacteria.

Difficulty in studying *Ehrlichia* species pathogenesis is partly due to their nature as obligate intracellular bacteria. Although they can be grown in tick and dog monocyte cell lines (Munderloh et al. 1995), they are challenging to maintain in culture and have complex nutritional requirements (Walker and Dumler 1996). It is believed that following inoculation by an infected tick into a new host during the blood-meal, these gram-negative, nonmotile bacteria are engulfed by circulating monocytes or neutrophils, and survive phagocytosis by inhibiting phagosome-lysosome fusion (Massung et al. 2002). This leads to the formation of characteristic membrane-bound, intracytoplasmic bacterial aggregates termed "morulae" (McQuiston et al. 1999). Within these morulae, *Ehrlichia* replicate rapidly, causing the infected cell to lose structural integrity and lyse, releasing thousands of infective ehrlichial particles that go on to infect new cells. In patients with active infection, these ehrlichial inclusions can be seen within vacuoles of

circulating leukocytes, primarily monocytes in cases of HME and neutrophils in cases of HGE. Because of this, HGE may be more readily identified than HME due to the greater number of circulating neutrophils. In later stages of the disease, morulae may be observed in leukocyte precursors within bone marrow (Walker and Dumler 1996).

Symptoms of ehrlichiosis may include hematopoietic, immune and nervous system involvement. The disease can vary in severity from an infectious mononucleosis-like syndrome to a life-threatening illness. Early symptoms of infection have a very generalized nature with a typical incubation time of one to three weeks (Massung et al. 2002). Initially pathology may include fever, headache, leukopenia and lymphopenia (due to destruction of white blood cells), myalgia and elevated levels of liver enzymes, particularly aspartate aminotransferase. Patients may initially disregard infection since these non-specific symptoms resolve spontaneously and a rash at the site of the associated tick bite is typically absent. The disease may be underreported since many cases of ehrlichiosis resemble mild infectious mononucleosis-like syndromes that are self-resolving.

Untreated, ehrlichiosis can cause severe complications, particularly in immunocompromised patients, the young and elderly, and those suffering from co-infection with another pathogen (Walker and Dumler 1996). In such cases hospitalization is usually required, as initial symptoms subside and give way to involvement with other organ systems. Because *Ehrlichia* have such a high tropism for hemopoietic tissue, long-standing infections result in a variety of bone marrow abnormalities, including hypoplasia, normcellularity, and hypercellularity (Walker and

Dumler 1996). In cases in which treatment has been delayed, ehrlichiosis has caused kidney failure, adult respiratory distress syndrome, meningitis and various neurologic disorders, and disseminated intravascular coagulation (McQuiston et al. 1999). Massive destruction of immune cells renders patients susceptible to secondary infections, often involving opportunistic pathogens. Case fatality rates may be as high as 5% for HME and 10% for HGE (McQuiston et al. 1999).

Early diagnosis of infection with *Ehrlichia chaffeensis* or the HGE agent presents a challenge to the physician from initial symptoms alone. Fortunately, many patients may recall a recent tick bite, but many such cases may be misdiagnosed as Rocky Mountain spotted fever, a more common tick-borne disease with similar early-stage symptoms. At present, very limited molecular-based diagnostic tests and indirect immunofluorescent assays are used to confirm a suspected case (McQuiston et al. 1999). Often the best method of diagnosis is through blood analysis, where decreased numbers of leukocytes are observed and ehrlichial morulae may be seen (Walker and Dumler 1996). Cases of HME and HGE may be clinically indistinguishable, and microscopic or molecular analysis is required to identify the causative agent. Severity of the disease is dependent on several factors, including patient age, underlying health and infectious dosage. Ehrlichiae are susceptible to a broad spectrum of antibiotics; the drug of choice is usually doxycycline or tetracycline. Treatment and recovery are highly effective with early diagnosis and therapy. In more severe cases requiring hospitalization, treatment is administered intravenously, and some sequelae may remain following recovery. Like

Lyme disease, avoiding tick habitats and wearing protective clothing are the best forms of prevention. No vaccine is currently available for any form of ehrlichiosis.

There is currently no national surveillance program for ehrlichiosis in the United States, so incidence rates are not known. Since ehrlichiosis is thought to be rare, it is not a reportable disease in many states. Underreporting is probably widespread, due to the common misdiagnosis of the disease and the fact that many cases are mild and self-resolving. From 1986 to 1997, 1,223 cases of ehrlichiosis were reported from 30 different state health departments across the United States (McQuiston, 1999). Of these, 742 (60.7%) were identified as HME, 449 (36.7%) as HGE, and 32 (2.6%) as uncategorized (McQuiston et al. 1999). Most cases of HME have been reported from the southcentral and southeastern United States, particularly Arkansas, Missouri, North Carolina and Virginia. HGE has been most frequently reported in the northeast and several upper midwestern states, particularly Connecticut, New York, Minnesota and Wisconsin. At least three cases of HGE and two cases of HME have been identified in California. Two of the HGE cases occurred in Santa Cruz County (Barlough et al. 1997).

In areas of the United States where ehrlichiosis is endemic, *Amblyomma americanum* and *Ixodes scapularis* ticks are considered the primary vectors, and white-tailed deer, *Odocoileus virginianus*, and the white-footed mouse, *Peromyscus leucopus* are believed to be the most important reservoirs (Bakken and Dumler 2000). Prevalence studies of these agents in vector ticks remain limited, but estimates range anywhere from 10 to 90% (Barlough et al. 1997). However, in the west, the reservoirs are unknown, and prevalence estimates are based on several limited surveys (Barlough et al. 1997 and

Kramer et al. 1999). Recently, several studies have implicated the black-legged tick *Ixodes pacificus* as the primary vector for ehrlichiosis in California (Barlough et al. 1997 and Kramer et al. 1999). However, infection has also been demonstrated in the American dog tick, *Dermacentor variabilis*, but not in the Pacific coast tick, *Dermacentor occidentalis*. As with Lyme disease, the ability of these ticks to act as competent vectors for ehrlichiae is not known. Prevalence estimates of ehrlichiae in tick populations remains largely nonexistent across the state. With the identification of ehrlichiosis cases in Santa Cruz County, it is important to identify potential vectors and their infection prevalence in order to assess public risk.

Testing for Lyme Disease and Ehrlichiosis

Determining prevalence of *B. burgdorferi*, *E. chaffeensis* and the HGE agent in ticks collected from public-use recreation areas will elucidate the potential risk of human exposure to Lyme disease and ehrlichiosis. With its large deer population, damp coastal ranges, grasses and shrubbery, many parts of Santa Cruz County's parklands are ideal tick habitats. Due to the temperate climate, park visitation tends to be similar during the cooler winter months, when adult ticks are likely to be questing, to the summer months. This study represents the first widespread effort to establish prevalence estimates and distribution patterns of these tick-borne diseases among the adult tick populations of Santa Cruz County. Furthermore, it seeks to identify the potential vectors of *E. chaffeensis* and the HGE agent in Santa Cruz County, and to determine if co-infection with more than one of these etiological agents, including *B. burgdorferi*, is possible.

CHAPTER II

Distribution of *Borrelia burgdorferi*, *Ehrlichia chaffeensis* and the HGE agent in ticks (Acari: Ixodidae) from a coastal region of California

The following is a summarized report of the thesis research work, submitted to a peer-reviewed journal, *Journal of Medical Entomology*, for publication.

Journal of Medical Entomology
Articles
Holden et al.: Distribution of
Borrelia burgdorferi, *Ehrlichia*
chaffeensis and the HGE agent
in ticks (Acari: Ixodidae)
from a coastal region of California

John T. Boothby
Department of Biological Sciences
(408) 924-4850
jboothby@email.sjsu.edu

Kevin Holden¹, John T. Boothby¹, Sulekha Anand¹ and Robert F. Massung²
Department of Biological Sciences, San Jose State University, San Jose, California
95192¹; Division of Viral and Rickettsial Diseases, Centers for Disease Control and
Prevention, Atlanta, Georgia 30333²

ABSTRACT A study was conducted in Santa Cruz County to estimate the prevalence and distribution of the agents of Lyme disease, human granulocytic (HGE) and human monocytic (HME) ehrlichiosis in 1187 ixodid ticks collected from eight public-use recreation areas over a two-year period. *Borrelia burgdorferi*, the causative agent of Lyme disease, was detected by a polymerase chain reaction (PCR) in 44 of 776 (5.67%) *Ixodes pacificus* ticks and in 3 of 58 (5.17%) *Dermacentor variabilis* ticks. The HGE agent was detected by PCR in 48 (6.19%) *I. pacificus* ticks and 5 (8.62%) *D. variabilis* ticks. *Ehrlichia chaffeensis*, the causative agent of HME, was detected by nested PCR in just 5 (0.64%) *I. pacificus* ticks and 4 (6.9%) *D. variabilis* ticks. Interestingly, 8 (1.03%) *I. pacificus* ticks were co-infected with *B. burgdorferi* and the HGE agent, and just 1 (0.12%) with *B. burgdorferi* and *E. chaffeensis*. Less than one percent of 353 *Dermacentor occidentalis* ticks showed evidence of infection with any of the agents tested. To our knowledge, this is the first reported identification of the HGE agent and *E. chaffeensis* in *D. occidentalis* ticks from California. This study represents the first extensive survey of Lyme and the ehrlichial diseases across multiple areas of Santa Cruz County, and suggests that prevalence of *B. burgdorferi* in Santa Cruz County may be higher than other areas of the state.

KEY WORDS *Borrelia burgdorferi*, *Ehrlichia* sp., *Ixodes pacificus*, Lyme disease, ehrlichiosis, California

Lyme borreliosis (Lyme disease) is a multiphased, multisystem disease characterized by dermatological, musculoskeletal and neurological manifestations. The etiological agent, *Borrelia burgdorferi* sensu lato, has been identified in a variety of tick species across the globe, and is the most common tick-borne zoonosis in humans worldwide (Steere 1989). In California, the western black-legged tick, *Ixodes pacificus* is the primary vector (Lane et al. 1991). Despite recent studies implicating the nymphal stages as the primary human vector (Clover and Lane, 1995), greater than 40% of reported cases with erythema migrans in California (1991-1999) were acquired during the winter and spring months when nymphal stages are not thought to be highly active (Monsen et al. 1999). Other tick species native to the state may become infected with the spirochete, but several studies have shown that they act as poor vectors for the disease (Johns et al. 1998). The Centers for Disease Control and Prevention reported 1619 cases in California from 1990 to 1999 and designated several northwestern counties of the state as endemic. Previous studies, often using microscopic identification rather than more specific, sensitive molecular techniques, have estimated prevalence of 1-2% in adult ticks in selected areas of California (Lane et al. 1991, Kramer and Beesley 1993). However, prevalence estimates for many parts of this large and ecologically diverse state remain limited.

Human ehrlichioses are recently described tick-borne diseases caused by rickettsiae of the *Ehrlichia* genus that are closely related to pathogens of horses and domestic ruminants. Pathogenesis of these diseases is poorly understood, and manifestations include a wide array of symptoms involving the hematopoietic, immune

and nervous systems. Human monocytic ehrlichiosis (HME), caused by *Ehrlichia chaffeensis*, infects mononuclear leukocytes, and was first recognized in Arkansas in 1986 (Maeda et al. 1987), and in California in 1994 (Vugia et al. 1996). Human granulocytic ehrlichiosis (HGE) caused by an uncharacterized *Ehrlichia* species known as the HGE agent infects polymorphonuclear leukocytes, and was first described in Minnesota and Wisconsin in 1994 (Bakken et al. 1994) and in California in 1995 (Gewirtz et al. 1996). Although most cases of HME have been reported from the southern United States, and most cases of HGE from the midwestern and northeast, at least three cases of HGE and two cases of HME have been identified in California (California Department of Health Services). Two of the HGE cases occurred in Santa Cruz County (Barlough et al. 1997). Most cases of ehrlichiosis in horses occur during the winter and early spring months (Vreedevoe et al. 1995), correlating to the period of peak activity for adult *I. pacificus* ticks, which is implicated as the primary vector for these diseases in California (Barlough et al. 1997, Kramer et al. 1999). Infection has also been demonstrated in the American dog tick, *Dermacentor variabilis*, but not in the Pacific coast tick, *Dermacentor occidentalis* (Barlough et al. 1997, Kramer et al. 1999). The natural reservoirs of HME and HGE in California are not known (Barlough et al. 1997, Kramer et al. 1999), and prevalence estimates of *Ehrlichia* sp. in tick populations are limited.

Public use recreation areas of Santa Cruz County, with their large deer populations, damp coastal ranges, long grasses, and shrubbery make ideal habitats for ixodid ticks, and potentially represent locations of high risk of human exposure to tick

bites. Due to the temperate climate, public use of the over 20 state and county recreational areas can be heavy even during the cooler winter months, when adult ticks are likely to be questing. This study represents the first widespread effort to establish prevalence estimates and distribution patterns of *B. burgdorferi*, *E. chaffeensis* and the HGE agent among the adult tick populations of Santa Cruz County.

Materials and Methods

Tick collection. A total of 1187 adult *I. pacificus*, *D. occidentalis* and *D. variabilis* ticks were collected from eight public-use recreation areas in Santa Cruz County, California between January and May in 2000 and 2001. Collection sites (Big Basin State Park, Castle Rock State Park, DeLaveaga City Park, Forest of Nisene Marks State Park, Henry Cowell State Park, New Brighton State Beach, Sunset State Beach, Wilder Ranch State Park) were chosen in part due to their year-round public use. Questing ticks were collected from vegetation along the edges of well-defined hiking and game trails identified as potential tick habitats using a flannel flag. Ticks were stored individually in 70% ethanol until processing, and species, sex, location and collection date of each was recorded.

DNA extraction. Each tick DNA extraction was performed individually using the DNeasy tissue kit (Qiagen, Valencia, CA) following the manufacturer's recommended protocol, with minor modifications as follows: before extraction, ticks were removed from 70% ethanol, individually placed into 1.5-ml microtubes, and allowed to dry. Microtubes were immersed in liquid nitrogen for 15 minutes before

maceration in 180 µl of Buffer ATL (Qiagen) using sterile disposable microtube pestles (VWR, San Francisco, CA). After addition of 20 µl of proteinase K (Qiagen), samples were incubated at 55°C on a heat block for 3 hours with occasional vortexing, and DNA was extracted according to the manufacturer's protocol. The DNA was eluted once using 200 µl of Buffer AE (Qiagen) and the eluate reapplied to the purification column to maximize DNA concentration. All eluates were stored in 96-well format deep well blocks (Wheaton, Millville, NJ) at -20°C prior to polymerase chain reaction (PCR) analysis.

Bacterial cultures and DNA controls. *Borrelia burgdorferi* B31 cultures (ATCC 35210) were maintained in BSKII medium supplemented with rabbit serum at 33°C and passaged once per week (Barbour 1984). Cells were enumerated by dark-field microscopy in a Petroff-Hausser counting chamber. Total genomic DNA was extracted from these cultures using the DNeasy tissue kit (Qiagen) following the manufacturer's recommended protocol for bacterial cultures. These extracts served as positive controls for PCR. For the HGE agent and *E. chaffeensis*, previously isolated genomic DNA was obtained to serve as positive controls (CDC, Atlanta, GA).

Polymerase chain reaction. All 50 µl PCR reactions contained 10 pmoles of each primer (Invitrogen, Carlsbad, CA), 200 µM of each dNTP (Amersham Biosciences, Piscataway, NJ), 5 µl of 10X PCR buffer (100 mM Tris-HCl (pH 8.6), 500 mM KCl, 15 mM MgCl₂, 1% Triton X-100, Amersham), 5 µl of template, nuclease-free water (Invitrogen) and 2.5 units of *Taq* cloned hot start DNA polymerase (Amersham). For the amplification of *B. burgdorferi* DNA, primers specific for the flagellin gene, FL6F (5'

TTCAGGGTCTCAAGCGTCTTGGACT) and FL7R (5' GCATTTTCAATTTTAGCAA-GTGATG), were used to produce a 276-bp fragment (Picken 1992). Cycling conditions were 5-min at 95°C, then 30 cycles (1-min at 94°C, a 1-min at 55°C, and 2-min at 72°C) followed by 7-min at 72°C. Amplification of HGE DNA utilized primers specific for the p44 gene, MSP3F (5' CCAGCGTTTAGCAAGATAAGAG) and MSP3R (5' GCCCAGT-AACAACATCATAAGC), to produce a 334-bp fragment (Massung et al. 2002). Cycling conditions were 5-min at 95°C, then 40 cycles (1-min at 94°C, a 1-min at 55°C, and 2-min at 72°C) followed by 7-min at 72°C. *Ehrlichia chaffeensis* DNA was amplified using primers specific for the VLPT gene, FB3AF (5' AAGACTGAAACGTTATAGAG) and FB5AR (5' GTGACATCTTAGTTTAATAGAAC), which after a nested PCR produces four amplicons, a 396-bp (three tandem repeats), a 486-bp (four tandem repeats), a 576-bp (five tandem repeats) and a 666-bp (six tandem repeats) (Stromdahl et al. 1998). Nested PCR was performed using 0.5 µl of primary PCR product as template and 10 pmoles of each nested primer, FB3F (5' GCCTAATTCAGATAAACTAAC) and FB5CR (5' GTTGATCATGTACCTGTGTG). Cycling conditions were 5-min at 95°C, then 30 cycles (1-min at 94°C, a 1-min at 55°C, and 45-sec at 72°C) followed by 7-min at 72°C. For the nested reaction, annealing temperature was adjusted to 60°C.

Prior to testing tick DNA extracts, specificity and limits of detection (LOD) were established for each PCR assay by using titrations of control DNA as template for PCR. All amplifications were performed in a 96-well format using an MJ Research DNA Engine Tetrad cycler (MJ Research, Inc., Waltham, MA).

Gel electrophoresis. PCR products were diluted 10-fold in Blue Juice (Invitrogen) and subjected to electrophoresis in Vistra-Green (Amersham) stained 2% agarose gels. Images were captured using the Typhoon (Amersham) scanner. All presumptive positive reactions were confirmed with a second round of PCR and gel electrophoresis.

Results

During the months of January to May in 2000 and 2001, a total of 1187 ticks were collected from eight public recreational areas in Santa Cruz County (Table 1). These included 776 *I. pacificus* adults (370 females, 406 males), 353 *D. occidentalis* adults (162 females, 191 males) and 58 *D. variabilis* adults (24 females, 34 males). *Ixodes pacificus* and *D. occidentalis* ticks were collected from all eight sites, and *D. variabilis* were collected from five of the eight sites.

Each tick was subjected to individual PCR analysis for *B. burgdorferi*, *E. chaffeensis* and the HGE agent (Fig. 1). The *B. burgdorferi* assay was determined to have an LOD of 37 fg DNA, the HGE assay LOD was 135 fg of DNA, and the *E. chaffeensis* assay LOD was 350 fg of DNA. All primer pairs were reacted with positive and negative DNA control templates in order to confirm species specificity. *Borrelia burgdorferi* was detected in 44 of 776 (5.67%) *I. pacificus*, 3 of 58 (5.17%) *D. variabilis* and 3 of 353 (0.85%) *D. occidentalis* ticks (Table 2). The HGE agent was detected in 48 of 776 (6.19%) *I. pacificus*, 5 of 58 (8.62%) *D. variabilis* and 4 of 353 (1.53%) *D. occidentalis* ticks. *Ehrlichia chaffeensis* was detected in 5 of 776 (0.64%) *I. pacificus*, 4

of 58 (6.90%) *D. variabilis* and 1 of 353 (0.28%) *D. occidentalis* ticks. According to a Chi-square test of independence, male and female ticks did not differ significantly in rates of infection for *B. burgdorferi* ($\chi^2(1) = 2.08, p = 0.15$), *E. chaffeensis* ($\chi^2(1) = 1.31, p = 0.25$) or the HGE agent ($\chi^2(1) = 1.41, p = 0.24$). Furthermore, a separate Chi-square test of independence showed that a positive result was not dependent on the year or the sex of the tick collected ($\chi^2(1) = 0.03, p = 0.87$). Concentrations of extracted tick DNA averaged 21.96 ng/ μ l (range = 113.35, standard deviation = 13.61) for all species. Positives were successfully identified throughout the entire range of DNA extraction concentrations (Holden, 2002).

Co-infection with *B. burgdorferi* and *E. chaffeensis* was detected in *I. pacificus* (1 of 776, 0.12%), but not in *D. variabilis* and *D. occidentalis* ticks. Co-infection with *B. burgdorferi* and the HGE agent was detected in *I. pacificus* (8 of 776, 1.03%), but not in *D. variabilis* and *D. occidentalis* ticks. Finally, co-infection with *E. chaffeensis* and the HGE agent was not detected in any species.

Discussion

This study represents the first widespread effort to establish prevalence estimates and distribution patterns of tick-borne diseases among the adult tick populations in Santa Cruz County. We found evidence that the etiological agents of Lyme disease, HME and HGE are present in actively questing adult ticks in Santa Cruz County, and that these vectors may be infected with more than one of these pathogens.

Evidence of *B. burgdorferi* was found in 5.67% of 776 adult *I. pacificus* ticks collected in public parklands in Santa Cruz County, confirming previous findings that *I. pacificus* is the primary vector of Lyme borreliosis in California. This finding is notably higher than prevalence estimates reported elsewhere in the state (Lane et al. 1991, Kramer and Beesley 1993). Many areas of Santa Cruz County may provide a more suitable habitat for *I. pacificus* ticks and *B. burgdorferi* reservoirs than previously surveyed localities. *Ixodes pacificus* ticks are more likely to quest in locations that are humid and well shaded (Lane et al. 1995), and the damp, foggy coastal mountains and foothills of Santa Cruz County provide such a habitat. The protected parklands surveyed in our study may also provide optimal habitats for reservoir hosts, such as deer. However, our sampling transects were placed in areas of likely tick habitat, and areas of lower prevalence may have been overlooked.

In California important hosts of *I. pacificus* nymphs include the western fence lizard (*Sceloporus occidentalis*), which possesses a borreliacidal substance in its blood that eliminates spirochetes from the midgut of infected nymphal ticks (Lane and Quistad 1998). Since nymphs feeding on this lizard to repletion apparently lose their infections before or during the transtadial molt (Lane et al. 1995), the lower prevalence found in adult ticks in California compared to those surveyed in the northeastern United States can be explained. Our findings, demonstrating a higher prevalence in adult ticks from Santa Cruz County, may be due to sampling areas that provide poor habitats for these lizards. Although their distribution is considered to be widespread across California, little work has been done to determine their distribution and abundance.

In *D. variabilis* and *D. occidentalis* ticks, the prevalence of *B. burgdorferi* was 5.17% and 0.85%, respectively. However, since the number of *D. variabilis* ticks collected represented less than 5% of the total number of ticks (Table 1) compared to *I. pacificus* or *D. occidentalis* species, our prevalence estimate for this species should be considered with caution. A lower abundance of *D. variabilis* than other species in Santa Cruz County is consistent with previous studies (Furman and Loomis 1984). Although some *D. variabilis* ticks may contain *B. burgdorferi*, they probably act as poor vectors for this pathogen and may not sustain infection due to antimicrobial proteins in their hemolymph (Johns et al. 1998). Since *D. occidentalis* ticks demonstrated such a low prevalence, 0.85%, despite greater numbers collected, similar mechanisms may be active.

The HGE agent was found in 6.19% of adult *I. pacificus*, 8.62% of *D. variabilis* ticks and 1.03% of *D. occidentalis* ticks. To our knowledge, this is the first identification of the HGE agent in *D. occidentalis* ticks from California. However, the competency of *D. variabilis* and *D. occidentalis* ticks to act as vectors is speculative since these ticks may have the ability to eliminate HGE infection as they do with *B. burgdorferi*.

Conclusions regarding prevalence in *Dermacentor* sp. ticks should be guarded due to the smaller sample numbers and lower prevalence. These findings therefore confirm that *I. pacificus* ticks are the primary vectors for this agent in California (Kramer et al. 1999).

Ehrlichia chaffeensis occurred in 10 of 1187 ticks (*I. pacificus* 5 of 776, 0.64%, *D. variabilis* 4 of 58, 6.90% and *D. occidentalis* 1 of 353, 0.28%). To our knowledge, this is the first identification of *E. chaffeensis* in *D. occidentalis* ticks from California. These low prevalence estimates could be due to sampling areas that serve as poor habitats

for the unknown reservoirs that maintain this microbe, or may indicate that our PCR assay was not highly specific for genotypes found in California due to the lack of sequence information. The prevalence of this agent may in fact be lower in this geographical region than the HGE agent since our findings for *E. chaffeensis* in California are consistent with other published reports (Kramer et al. 1999). Although five *I. pacificus* ticks were positive for HME, the small sample size precludes determining the primary vector for this agent.

We found the prevalence of *B. burgdorferi* may be higher in Santa Cruz County than in other areas of the state, where prevalence of 1-2% has been reported (Lane et al. 1991, Kramer and Beesley 1993), but studies utilizing a greater number of samples, locations, and habitats for adult ticks and nymphs will be needed to confirm the levels of infection and the availability of competent reservoirs. We confirmed the presence of the HGE agent and *E. chaffeensis* in California, and identified the major vector of the HGE agent as *I. pacificus*. For the first time, we identified the HGE agent and *E. chaffeensis* in *D. occidentalis* ticks. Several ticks in this study supported more than one etiological agent. Our results indicate that *B. burgdorferi*, the HGE agent and *E. chaffeensis* are present in actively questing adult ticks in Santa Cruz County, and represent a health risk in public recreation areas.

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Table 1. Number of adult ticks, by species, collected from public recreation areas in Santa Cruz County, 2000-2001.

Location	No. of <i>I. pacificus</i> (%) ¹	No. of <i>D. occidentalis</i> (%) ¹	No. of <i>D. variabilis</i> (%) ¹	Total (%) ²
Big Basin SP	113 (75.84%)	33 (22.15%)	3 (2.01%)	149 (12.55%)
Castle Rock SP	264 (70.40%)	104 (27.73%)	7 (1.87%)	375 (31.59%)
DeLaveaga CP	91 (75.21%)	30 (24.79%)	0	121 (10.19%)
Forest of Nisene Marks SP	28 (73.68%)	4 (10.53%)	6 (15.79%)	38 (3.20%)
Henry Cowell SP	133 (54.73%)	101 (41.56%)	9 (3.71%)	243 (20.47%)
New Brighton SB	85 (80.19%)	21 (19.81%)	0	106 (8.93%)
Sunset SB	6 (28.57%)	15 (71.43%)	0	21 (1.77%)
Wilder Ranch SP	56 (41.79%)	45 (33.58%)	33 (24.63%)	134 (11.30%)
All Locations	776 (65.37%) ²	353 (29.74%) ²	58 (4.89%) ²	1187 (100%)

SP, State Park; CP, City Park; SB, State Beach. ¹Percent of ticks collected within each location. ²Percent of all ticks collected.

Table 2. Prevalence and distribution of *Borrelia burgdorferi*, the HGE agent and *Ehrlichia chaffeensis* in adult *Ixodes pacificus*, *Dermacentor occidentalis* and *Dermacentor variabilis* ticks.

Location Pathogen(s)	No. of positive <i>I. pacificus</i> (%) ^a	No. of positive <i>D. occidentalis</i> (%) ^a	No. of positive <i>D. variabilis</i> (%) ^a	Total (%) ^b
All Locations				
<i>B. burgdorferi</i>	44 (5.67%)	3 (0.85%)	3 (5.17%)	50 (4.21%)
HGE agent	48 (6.19%)	4 (1.13%)	5 (8.62%)	57 (4.80%)
<i>E. chaffeensis</i>	5 (0.64%)	1 (0.28%)	4 (6.90%)	10 (0.84%)
<i>B. burgdorferi</i> + HGE agent	8 (1.03%)	0	0	8 (0.67%)
<i>B. burgdorferi</i> + <i>E. chaffeensis</i>	1 (0.13%)	0	0	1 (0.08%)
<i>E. chaffeensis</i> + HGE agent	0	0	0	0
Big Basin SP				
<i>B. burgdorferi</i>	7 (6.19%) ¹	0	0	7 (4.70%)
HGE agent	5 (4.42%) ¹	2 (6.06%)	0	7 (4.70%)
<i>E. chaffeensis</i>	0	0	0	0
Castle Rock SP				
<i>B. burgdorferi</i>	13 (4.92%) ^{2,3}	1 (0.96%)	1 (14.29%)	15 (4.00%)
HGE agent	13 (4.92%) ²	1 (0.96%)	0	14 (3.73%)
<i>E. chaffeensis</i>	2 (0.76%) ³	0	1 (14.29%)	3 (0.80%)
DeLaveaga CP				
<i>B. burgdorferi</i>	4 (4.40%) ⁴	0	0	4 (3.31%)
HGE agent	5 (5.49%) ⁴	0	0	5 (4.13%)
<i>E. chaffeensis</i>	0	0	0	0
Forest of Nisene Marks SP				
<i>B. burgdorferi</i>	5 (17.86%) ⁵	0	0	5 (13.16%)
HGE agent	3 (10.71%) ⁵	0	0	3 (7.89%)
<i>E. chaffeensis</i>	0	0	1 (16.67%)	1 (2.63%)
Henry Cowell SP				
<i>B. burgdorferi</i>	7 (5.26%)	1 (0.99%)	0	8 (3.29%)
HGE agent	9 (6.77%)	1 (0.99%)	0	10 (4.12%)
<i>E. chaffeensis</i>	3 (2.26%)	1 (0.99%)	1 (11.11%)	5 (2.06%)
New Brighton SB				
<i>B. burgdorferi</i>	5 (5.88%) ⁶	0	0	5 (4.72%)
HGE agent	10 (11.76%) ⁶	0	0	10 (9.43%)
<i>E. chaffeensis</i>	0	0	0	0
Sunset SB				
<i>B. burgdorferi</i>	0	0	0	0
HGE agent	1 (16.67%)	0	0	1 (4.76%)
<i>E. chaffeensis</i>	0	0	0	0
Wilder Ranch SP				
<i>B. burgdorferi</i>	3 (5.36%)	1 (2.22%)	2 (6.06%)	6 (4.48%)
HGE agent	2 (3.57%)	0	5 (15.15%)	7 (5.22%)
<i>E. chaffeensis</i>	0	0	1 (3.03%)	1 (0.75%)

SP, State Park; CP, City Park; SB, State Beach. ¹Two *I. pacificus* ticks with *B. burgdorferi* + HGE agent. ^{2,3}Three *I. pacificus* ticks with *B. burgdorferi* + HGE agent; and one with *B. burgdorferi* + *E. chaffeensis*. ^{4,5,6}One *I. pacificus* tick with *B. burgdorferi* + HGE agent. ^aPercent of this tick species positive for this agent at this location(s). ^bPercent of all tick species positive for this agent at this location(s).

Figure 1. Typical 2% agarose gel analysis of *Borrelia burgdorferi* (A), HGE agent (B), and *Ehrlichia chaffeensis* (C) polymerase chain reaction (PCR) products using DNA isolated from ticks. Lane 1 (A) contains a 100 bp to 1.5 kb DNA size ladder (Invitrogen), lanes 1 and 2 (B) contain 100 bp to 12 kb and 100 bp to 1.5 kb DNA size ladders respectively and lane 1 (C) contains a 100 bp to 12kb DNA size ladder. Lanes 3-26 contain individual tick PCR reactions. Positive reactions are indicated by a 276-bp product for *Borrelia burgdorferi* (A, lane 7), a 334-bp product for HGE agent (B, lane 8), and 396-bp, 486-bp, 576-bp and 666-bp products for *Ehrlichia chaffeensis* (C, lane 14). All other reactions are negative.

CHAPTER III

Additional Methodology and Expanded Results and Discussion

Additional Materials and Methods

A total of 1187 adult *I. pacificus*, *D. occidentalis* and *D. variabilis* ticks were collected between January and May 2000 and 2001 from eight public-use recreation areas in Santa Cruz County, California. The eight collection sites included: Big Basin State Park, Castle Rock State Park, DeLaveaga City Park, Forest of Nisene Marks State Park, Henry Cowell State Park, New Brighton State Beach, Sunset State Beach, and Wilder Ranch State Park (Appendix A). The locations of these trails were recorded on topographical maps using TOPO! computer software (Wildflower Productions, San Francisco, CA) and global positioning satellite (GPS) coordinate data recorded at the sites (Appendix B-K). Each collection site was visited twice yearly with the exception of Forest of Nisene Marks State Park (visited in 2001 only) and DeLaveaga City Park (visited only once in 2000, twice in 2001), and all collections took place during the daytime over a period of two hours.

Following collection, ticks were stored in 70% ethanol in individual wells of deep-well 96 well blocks (Wheaton) until further processing. Each tick was subjected to individual PCR analysis for *B. burgdorferi*, *E. chaffeensis* and the HGE agent. Reactions were carried out in 96-well plates (MJ Research). For each PCR plate, a gel dilution plate was prepared using 2 µl of PCR product and 18 µl of Blue Juice (Invitrogen). All

20 µls were loaded into Vistra-Green stained 2% agarose gels and electrophoresised for 45 minutes at 90 volts. Each gel was cast with 192 wells in order to accommodate two 96-well PCR plates and appropriate DNA size ladders.

Expanded Results

A catalog was created assigning each tick a unique accession number, sex, species, location and date collected, DNA concentration following extraction, and PCR results were noted. *Ixodes pacificus* and *D. occidentalis* ticks were collected from each park, and *D. variabilis* were collected from five of the eight sites (Chapter II, Table 1). Castle Rock State Park yielded the most ticks, with 375, while Sunset State Beach yielded the least, with 21 (Chapter II, Table 1). Of the 1187 ticks collected, 776 were *I. pacificus*, 353 were *D. occidentalis*, and 58 were *D. variabilis*. A Chi-square test of independence analysis showed that male and female ticks did not differ significantly in rates of infection for *B. burgdorferi* ($\chi^2(1) = 2.08, p = 0.15$), *E. chaffeensis* ($\chi^2(1) = 1.31, p = 0.25$) or the HGE agent ($\chi^2(1) = 1.41, p = 0.24$). Furthermore, a separate Chi-square test of independence showed that a positive result was not dependent on the year or the sex of the tick collected ($\chi^2(1) = 1.06, p = 0.87$).

The average extracted DNA concentration was 21.96 ng/µl (range = 113.35, standard deviation = 13.61) for all ticks; 26.85 ng/µl (range = 112.95, standard deviation = 13.62) for *D. occidentalis*; 32.16 ng/µl (range = 84.00, standard deviation = 17.07) for *D. variabilis*; and 18.97 ng/µl (range = 59.70, standard deviation = 12.25) for *I. pacificus*. The average extracted DNA concentration was 18.43 ng/µl (range = 59.25, standard

deviation = 12.47) for all ticks positive for any pathogen; 22.53 ng/μl (range = 25.60, standard deviation = 7.95) for positive *D. occidentalis*; 29.05 ng/μl (range = 53.85, standard deviation = 16.29) for positive *D. variabilis*; and 16.61 ng/μl (range = 59.25, standard deviation = 12.47) for positive *I. pacificus*. The distribution of positive ticks extended throughout the range of measured DNA concentrations, from 1.25 ng/μl to 60.50 ng/μl (Appendix L).

A total of 21 gels were imaged using a Typhoon (Amersham) scanner. Typical results can be seen in Chapter II, Figure 1. Suspected positives were identified (Appendix N) and confirmed with a second round of PCR and gel electrophoresis (Appendix M).

Overall, *B. burgdorferi* was detected in 50 of 1187 (4.21%) ticks (Chapter II, Table 2). By tick species, *B. burgdorferi* was detected in *I. pacificus* (44 of 776, 5.67%), *D. variabilis* (3 of 58, 5.17%) and *D. occidentalis* (3 of 353, 0.85%) ticks. *Ehrlichia chaffeensis* was detected in 10 of 1187 (0.84%) ticks. By tick species, *E. chaffeensis* was detected in *I. pacificus* (5 of 776, 0.64%), *D. variabilis* (4 of 58, 6.90%) and *D. occidentalis* (1 of 353, 0.28%) ticks. The HGE agent was detected in 57 of 1187 (4.80%) ticks. By tick species, the HGE agent was detected in *I. pacificus* (48 of 776, 6.19%), *D. variabilis* (5 of 58, 8.62%) and *D. occidentalis* (4 of 353, 1.53%) ticks. Co-infection with *B. burgdorferi* and *E. chaffeensis* was detected in *I. pacificus* (1 of 776, 0.12%), but not in *D. variabilis* and *D. occidentalis* ticks. Co-infection with *B. burgdorferi* and the HGE agent was detected in *I. pacificus* (8 of 776, 1.03%), but not in *D. variabilis* and *D.*

occidentalis ticks. Co-infection with *E. chaffeensis* and the HGE agent was not detected in any species (Chapter II, Table 2).

In Big Basin State Park, *B. burgdorferi* was detected in 7 of 149 (4.70%) ticks (Chapter II, Table 2). By tick species, *B. burgdorferi* was detected in *I. pacificus* (7 of 113, 6.19%), but not in *D. variabilis* and *D. occidentalis* ticks. *Ehrlichia chaffeensis* was not detected in any tick species. The HGE agent was detected in 7 of 149 (4.70%) ticks. By tick species, the HGE agent was detected in *I. pacificus* (5 of 113, 4.42%) and *D. occidentalis* (2 of 33, 6.06%) but not in *D. variabilis* ticks. Co-infection with *B. burgdorferi* and *E. chaffeensis* was not detected in any tick species. Co-infection with *B. burgdorferi* and the HGE agent was detected in *I. pacificus* (2 of 113, 1.17%), but not in *D. variabilis* and *D. occidentalis* ticks. Co-infection with *E. chaffeensis* and the HGE agent was not detected in any species (Chapter II, Table 2).

In Castle Rock State Park, *B. burgdorferi* was detected in 15 of 375 (4.00%) ticks (Chapter II, Table 2). By tick species, *B. burgdorferi* was detected in *I. pacificus* (13 of 264, 4.92%), *D. variabilis* (1 of 7, 14.29%) and *D. occidentalis* (1 of 104, 0.96%) ticks. *Ehrlichia chaffeensis* was detected in 3 of 375 (0.80%) ticks. By tick species, *E. chaffeensis* was detected in *I. pacificus* (2 of 264, 0.76%) and *D. variabilis* (1 of 7, 14.29%) but not in *D. occidentalis* ticks. The HGE agent was detected in 14 of 375 (3.73%) ticks. By tick species, the HGE agent was detected in *I. pacificus* (13 of 264, 4.92%) and *D. occidentalis* (1 of 104, 0.96%) but not in *D. variabilis* ticks. Co-infection with *B. burgdorferi* and *E. chaffeensis* was detected in *I. pacificus* (1 of 264, 0.38%), but not in *D. variabilis* and *D. occidentalis* ticks. Co-infection with *B. burgdorferi* and the

HGE agent was detected in *I. pacificus* (3 of 264, 1.14%), but not in *D. variabilis* and *D. occidentalis* ticks. Co-infection with *E. chaffeensis* and the HGE agent was not detected in any species (Chapter II, Table 2).

In DeLaveaga City Park, *B. burgdorferi* was detected in 4 of 121 (3.31%) ticks (Chapter II, Table 2). By tick species, *B. burgdorferi* was detected in *I. pacificus* (4 of 91, 4.40%), but not in *D. variabilis* and *D. occidentalis* ticks. *Ehrlichia chaffeensis* was not detected in any tick species. The HGE agent was detected in 5 of 121 (4.13%) ticks. By tick species, the HGE agent was detected in *I. pacificus* (5 of 121, 4.13%), but not in *D. variabilis* and *D. occidentalis* ticks. Co-infection with *B. burgdorferi* and *E. chaffeensis* was not detected in any tick species. Co-infection with *B. burgdorferi* and the HGE agent was detected in *I. pacificus* (1 of 91, 1.10%), but not in *D. variabilis* and *D. occidentalis* ticks. Co-infection with *E. chaffeensis* and the HGE agent was not detected in any species (Chapter II, Table 2).

In Forest of Nisene Marks State Park, *B. burgdorferi* was detected in 5 of 38 (13.16%) ticks (Chapter II, Table 2). By tick species, *B. burgdorferi* was detected in *I. pacificus* (5 of 38, 13.16%), but not in *D. variabilis* and *D. occidentalis* ticks. *Ehrlichia chaffeensis* was detected in 1 of 38 (2.63%) ticks. By tick species, *E. chaffeensis* was detected in *D. variabilis* (1 of 6, 16.67%), but not in *I. pacificus* and *D. occidentalis* ticks. The HGE agent was detected in 3 of 38 (7.89%) ticks. By tick species, the HGE agent was detected in *I. pacificus* (3 of 38, 7.89%), but not in *D. variabilis* and *D. occidentalis* ticks. Co-infection with *B. burgdorferi* and *E. chaffeensis* was not detected in any tick species. Co-infection with *B. burgdorferi* and the HGE agent was detected in *I. pacificus*

(1 of 38, 3.57%), but not in *D. variabilis* and *D. occidentalis* ticks. Finally, co-infection with *E. chaffeensis* and the HGE agent was not detected in any species (Chapter II, Table 2).

In Henry Cowell State Park, *B. burgdorferi* was detected in 8 of 243 (3.29%) ticks (Chapter II, Table 2). By tick species, *B. burgdorferi* was detected in *I. pacificus* (7 of 131, 5.26%) and *D. occidentalis* (1 of 101, 0.99%) but not in *D. variabilis* ticks. *Ehrlichia chaffeensis* was detected in 5 of 243 (2.06%) ticks. By tick species, *E. chaffeensis* was detected in *I. pacificus* (3 of 131, 2.26%), *D. variabilis* (1 of 9, 11.11%) and *D. occidentalis* (1 of 101, 0.99%) ticks. The HGE agent was detected in 10 of 1187 (4.12%) ticks. By tick species, the HGE agent was detected in *I. pacificus* (9 of 131, 6.77%) and *D. occidentalis* (1 of 101, 0.99%) but not in *D. variabilis* ticks. Co-infection of any combination of agents was not found in any tick species (Chapter II, Table 2).

In New Brighton State Beach, *B. burgdorferi* was detected in 5 of 106 (4.72%) ticks (Chapter II, Table 2). By tick species, *B. burgdorferi* was detected in *I. pacificus* (5 of 85, 5.88%), but not in *D. variabilis* and *D. occidentalis* ticks. *Ehrlichia chaffeensis* was not detected in any tick species. The HGE agent was detected in 10 of 106 (9.43%) ticks. By tick species, the HGE agent was detected in *I. pacificus* (10 of 85, 5.88%) but not *D. variabilis* and *D. occidentalis* ticks. Co-infection with *B. burgdorferi* and *E. chaffeensis* was not detected in any tick species. Co-infection with *B. burgdorferi* and the HGE agent was detected in *I. pacificus* (1 of 85, 1.18%), but not in *D. variabilis* and *D. occidentalis* ticks. Finally, co-infection with *E. chaffeensis* and the HGE agent was not detected in any species (Chapter II, Table 2).

In Sunset State Beach, *B. burgdorferi* and *E. chaffeensis* were not detected in any tick species (Chapter II, Table 2). The HGE agent was detected in 1 of 21 (4.76%) ticks. By tick species, the HGE agent was detected in *I. pacificus* (1 of 6, 16.67%), but not in *D. variabilis* and *D. occidentalis* ticks. Co-infection of any combination of agents was not found in any tick species (Chapter II, Table 2).

In Wilder Ranch State Park, *B. burgdorferi* was detected in 6 of 134 (4.48%) ticks (Chapter II, Table 2). By tick species, *B. burgdorferi* was detected in *I. pacificus* (3 of 56, 5.36%), *D. variabilis* (2 of 33, 5.36%) and *D. occidentalis* (1 of 45, 2.22%) ticks. *Ehrlichia chaffeensis* was detected in 1 of 134 (0.75%) ticks. By tick species, *E. chaffeensis* was detected in *D. variabilis* (1 of 33, 3.03%), but not in *I. pacificus* and *D. occidentalis* ticks. The HGE agent was detected in 7 of 134 (5.22%) ticks. By tick species, the HGE agent was detected in *I. pacificus* (2 of 56, 3.57%) and *D. variabilis* (5 of 33, 15.15%), but not in *D. occidentalis* ticks. Co-infection of any combination of agents was not found in any tick species (Chapter II, Table 2).

Expanded Discussion

This study was undertaken to determine the prevalence of several tick-borne pathogens in Santa Cruz County and their distribution among tick vector species. To our knowledge, this is the most comprehensive study of its kind conducted in the county despite recently described cases of Lyme disease and ehrlichiosis. We found evidence that the etiological agents of Lyme disease, HME and HGE are present in actively

questing adult ticks in Santa Cruz County, and that these vectors may be infected with more than one pathogen.

The prevalence of *B. burgdorferi* was found to be 5.67% in 776 adult *I. pacificus* ticks collected in public parklands in Santa Cruz County. *Ixodes pacificus* is considered the primary vector of Lyme borreliosis in California (Kramer et al. 1999). This infection rate is considerably higher than the 1-2% rate previously reported in adult *I. pacificus* from different areas of the state (Lane et al. 1991, Kramer and Beesley 1993). There are several possible explanations for this disparity. Many areas of Santa Cruz County may provide a more suitable habitat for *I. pacificus* ticks and *B. burgdorferi* reservoirs than previously surveyed localities. *Ixodes pacificus* ticks are more likely to quest in locations that are humid and well shaded (Lane et al. 1995), and the damp, foggy coastal mountains and foothills of Santa Cruz County provide such a habitat. Furthermore, the high prevalence of *B. burgdorferi* in *I. pacificus* ticks may result from the numerous protected parklands in the county that support adequate populations of reservoir hosts, such as deer.

Alternatively, since our collections took place in areas of likely tick habitat, other areas of lower prevalence may not be represented. For instance, Forest of Nisene Marks (Chapter II, Table 2) showed a much higher prevalence rate (17.86% in *I. pacificus* ticks) than the other parks surveyed. This may be explained by the smaller sample size of ticks that were collected from this location in comparison to others (only 28 *I. pacificus* ticks, Chapter II, Table 1), or that the collection areas contained focal points of ticks infected with Lyme disease. In Castle Rock State Park, where the most samples were collected (375 of 1187 ticks, with 264 *I. pacificus*), the prevalence of *B. burgdorferi* in *I. pacificus*

was 4.92% (Chapter II, Table 2). However, at Sunset State Beach (Chapter II, Table 2), none of the ticks were positives for *B. burgdorferi*. These differences may be due to the vastly different microhabitats observed between the different parks surveyed. While most of the parks contained damp, wooded regions and grassy plains, Sunset State Beach is a more arid environment with limited tree cover and most likely lower reservoir host populations. Perhaps a random sample might find the true prevalence of *B. burgdorferi* in *I. pacificus* ticks to be less than the 5.67% found on average through all parks in this study.

In California, important hosts of nymphal-stage ticks include small rodents and the western fence lizard (*S. occidentalis*), that may possess a borreliacidal substance in its blood that eliminates spirochetes from the midgut of infected nymphal ticks feeding upon it (Lane and Quistad 1998). Nymphs feeding on this lizard to repletion apparently lose their infections before or during the transtadial molt (Lane et al. 1995) explaining the lower prevalence found in adult ticks in California compared to those surveyed in the northeastern United States. One explanation of our findings of higher prevalence in adult ticks from Santa Cruz County, may be that our sample areas provide poor habitats for these lizards. Although their distribution is considered to be widespread across California, little work has been done to determine their distribution and abundance. An interesting future study would be to examine the habitats of these lizards in greater detail, and to create estimates of their populations within Santa Cruz County, and other areas of the state that appear to have relatively high prevalence rates.

In *D. variabilis* and *D. occidentalis* ticks the prevalence of *B. burgdorferi* was 5.17% and 0.85% respectively. These estimates represent presumptive rates since few *D. variabilis* ticks were collected (58 of 1187) in comparison to other species. According to our data, *D. variabilis* are not as relative abundant as *I. pacificus* or *D. occidentalis* in Santa Cruz County. Furthermore, several studies have shown that although *D. variabilis* ticks may contain *B. burgdorferi*, they may act as poor vectors for these pathogens and not retain infection for sustained periods (Johns et al. 1998). Antimicrobial proteins in *D. variabilis* hemolymph may limit infection with *B. burgdorferi*. Since *D. occidentalis* ticks demonstrated such a low prevalence (0.85%) despite higher numbers collected (353) similar mechanisms may be active.

The HGE agent was found in 6.19% of adult *I. pacificus*, 8.62% of *D. variabilis* ticks and 1.03% of *D. occidentalis* ticks. To our knowledge, this is the first identification of the HGE agent in *D. occidentalis* ticks from California. However, the competency of *D. variabilis* and *D. occidentalis* ticks to acts as vectors is speculative since these ticks may have the ability to eliminate HGE infection as they do with *B. burgdorferi*. These findings therefore confirm that *I. pacificus* ticks are the primary vectors for this agent in California (Kramer et al. 1999). The 6.19% prevalence rate in *I. pacificus* ticks coincides with previously published reports of 4.7% and 2.7% infection rates in neighboring counties (Kramer et al. 1999). However, conclusions regarding prevalence in *Dermacentor* sp. ticks should be guarded due to the smaller sample numbers and lower numbers of positives. Much like the findings for *B. burgdorferi*, parks appear to show variable prevalence rates possibly due to differences in microhabitats between the

collection sites and the suitability of these areas to act as good habitats for the required reservoir organisms. Interestingly, the prevalence of the HGE agent is slightly higher in both New Brighton State Beach and Wilder Ranch State Parks (Chapter II, Table 2), both areas where large numbers of ticks were collected (106 and 134 respectively). This anomaly might be explained by the heavy use by horse riders in these parks (personal observation), since horses are potential reservoirs for this pathogen in California (Vreedevoe et al. 1999).

Ehrlichia chaffeensis was identified in just 10 of 1187 ticks, including 5 of 776 *I. pacificus* (0.64%), 4 of 58 *D. variabilis* (6.90%) and 1 of 353 *D. occidentalis* (0.28%). To our knowledge, this is the first identification of *E. chaffeensis* in *D. occidentalis* ticks from California. The low prevalence observed in all ticks collected may be due to sampling habitats low in the (unknown) reservoir populations that maintain this microbe, or the primer specificity in the PCR assay used to test the ticks. The PCR targets used to test for this agent are not well developed, due to limited sequence information, and may not be highly specific for genotypes found in California. Still, these low prevalence findings may accurately reflect the abundance of *E. chaffeensis* in Santa Cruz County, since published reports note that HME is less common than HGE in California (Kramer et al. 1999). Although five *I. pacificus* ticks were positive for HME, other vectors may be active. Identification of *E. chaffeensis* in *D. variabilis* ticks must be considered presumptive since the sample number for this tick is small and its role as a competent vector is questionable.

Nine of 1187 ticks collected were co-infected with more than one pathogen, supporting previous observations that ticks in California are capable of harboring multiple disease agents (Barlough et al. 1997). All co-infected ticks were *I. pacificus*, indicating that *Dermacentor* sp. ticks may limit infection. Eight (0.67%) *I. pacificus* ticks were found to be co-infected with *B. burgdorferi* and the HGE agent while only one tick (0.08%) was found to be co-infected with *B. burgdorferi* and *E. chaffeensis*. Tick vectors harboring multiple pathogens may increase the human health risk since a single bite may transmit more than one pathogenic agent.

This study estimates prevalence and distribution patterns of several tick-borne pathogens among the adult tick populations of Santa Cruz County. We found the prevalence of *B. burgdorferi* to be higher in Santa Cruz County than what has been reported in other areas of the state. We confirmed the presence of the HGE agent and *E. chaffeensis* in California, and identified the major vector of the HGE agent as *I. pacificus*. For the first time, we identified the HGE agent and *E. chaffeensis* in *D. occidentalis* ticks. We provided further evidence that ticks in California can be infected with more than one pathogen. Since we found few *E. chaffeensis* within ticks, more work should be done to evaluate the vectors for this pathogen in California. Future surveys which include more extensive sampling of geographic areas, reservoir hosts, tick species, and different stages of tick life cycles will provide a more accurate picture of the true prevalence rates and vectors of these agents in the western United States. Our results indicate that these pathogens are present in actively questing adult ticks in Santa Cruz County parks, and represent a health risk to the general public.

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APPENDIX A

Tick Collection Sites in Santa Cruz County, California

Tick collection sites at state parks and beaches in Santa Cruz County, California.

APPENDIX B

Big Basin State Park (west) collection sites

Topographical map of Big Basin State Park (west) showing tick collection transects and dates: 4/2/00 and 4/9/00, Big Basin 1 (N 37° 05' 41", W 122° 16' 32") to Big Basin 2 (N 37° 05' 46", W 122° 16' 22"), 0.22 miles, due northeast.

APPENDIX C

Big Basin State Park (east) collection sites

Topographical map of Big Basin State Park (east) showing tick collection transects and dates: 4/17/01 and 4/22/01, Big Basin 3 (N 37° 09' 52", W 122° 11' 26") to Big Basin 4 (N 37° 09' 43", W 122° 11' 19"), 0.20 miles, due northwest.

APPENDIX D

Castle Rock State Park (east) collection sites

Topographical map of Castle Rock State Park (east) showing tick collection transects and dates: 2/6/00 and 2/17/00, Castle Rock 1 (N 37° 13' 07", W 122° 04' 28") to Castle Rock 2 (N 37° 12' 56", W 122° 04' 09"), 0.34 miles, due northwest.

APPENDIX E

Castle Rock State Park (west) collection sites

Topographical map of Castle Rock State Park (west) showing tick collection transects and dates: 2/19/01 and 3/24/01, Castle Rock 3 (N 37° 15' 08", W 122° 08' 11") to Castle Rock 4 (N 37° 15' 05", W 122° 08' 29"), 0.29 miles, due west; 5/29/01, Castle Rock 5 (N 37° 15' 01", W 122° 08' 11") to Castle Rock 6 (N 37° 14' 59", W 122° 08' 09"), 0.14 miles, due southeast.

APPENDIX F

DeLaveaga City Park collection sites

Topographical map of DeLaveaga City Park showing tick collection transects and dates: 5/7/00, DeLaveaga 1 (N 37° 00' 29", W 122° 00' 21") to DeLaveaga 2 (N 37° 00' 34", W 122° 00' 17"), 0.13 miles, due northeast; 2/4/01, DeLaveaga 3 (N 37° 00' 20", W 121° 59' 52") to DeLaveaga 4 (N 37° 00' 28", W 121° 59' 57"), 0.18 miles, due northwest; 3/6/01, DeLaveaga 5 (N 37° 00' 16", W 121° 59' 51") to DeLaveaga 6 (N 37° 00' 19", W 122° 00' 04"), 0.20 miles, due west.

APPENDIX G

Forest of Nisene Marks State Park collection sites

Topographical map of Forest of Nisene Marks State Park (east) showing tick collection transects and dates: 5/10/01, Nisene Marks 1 (N 36° 59' 24", W 121° 54' 04") to Nisene Marks 2 (N 36° 59' 39", W 121° 54' 09"), 0.14 miles, due northwest; 5/24/01, Nisene Marks 3 (N 36° 59' 21", W 121° 53' 52") to Nisene Marks 4 (N 36° 59' 37", W 121° 53' 22"), 0.21 miles, due north.

Henry Cowell State Park collection sites

Topographical map of Henry Cowell State Park showing tick collection transects and dates: 1/7/00 and 1/29/00, Henry Cowell 1 (N 37° 02' 36", W 122° 04' 21") to Henry Cowell 2 (N 37° 02' 37", W 122° 04' 10"), 0.11 miles, due west; 2/24/01 and 3/15/01, Henry Cowell 3 (N 37° 02' 35", W 122° 03' 58") to Henry Cowell 4 (N 37° 02' 31", W 122° 04' 03"), 0.19 miles, due south.

APPENDIX I

New Brighton State Beach collection sites

Topographical map of New Brighton State Beach showing tick collection transects and dates: 3/4/00 and 1/28/01, New Brighton 1 (N 36° 58' 50", W 121° 55' 41") to New Brighton 2 (N 36° 58' 44", W 121° 55' 44"), 0.15 miles, due southwest; 3/11/01 and 4/22/01, New Brighton 3 (N 36° 58' 48", W 121° 55' 46") to New Brighton 4 (N 36° 58' 45", W 121° 55' 51"), 0.14 miles, due southwest.

APPENDIX J

Sunset State Beach collection sites

Topographical map of Sunset State Beach showing tick collection transects and dates: 5/7/00 and 5/13/00, Sunset 1 (N 36° 52' 47", W 121° 49' 36") to Sunset 2 (N 36° 52' 39", W 121° 49' 33"), 0.14 miles, due southeast; 4/10/01 and 4/22/01, Sunset 3 (N 36° 52' 51", W 121° 49' 40") to Sunset 4 (N 36° 52' 57", W 121° 49' 42"), 0.12 miles, due northwest.

APPENDIX K

Wilder Ranch State Park collection sites

Topographical map of Wilder Ranch State Park showing tick collection transects and dates: 3/18/00 and 3/25/00, Wilder 1 (N 36° 57' 56", W 122° 06' 11") to Wilder 2 (N 36° 58' 00", W 122° 06' 04"), 0.16 miles, due east; 3/19/01 and 4/12/01, Wilder 3 (N 36° 57' 53", W 122° 06' 16") to Wilder 4 (N 36° 57' 40", W 122° 05' 14"), 0.13 miles, due northeast.

APPENDIX L

Distribution of tick DNA extracts positive or negative for *Borrelia burgdorferi*,
Ehrlichia chaffeensis and the HGE agent

Distribution of tick DNA extracts positive or negative for *Borrelia burgdorferi*, *Ehrlichia chaffeensis* and the HGE agent. Numbers above bars represent the number of ticks positive (red) for at least one pathogen, or negative (black) for all pathogens, within the stated tick DNA concentration range.

APPENDIX M

Confirmation of ticks presumptive for tick-borne pathogens by gel electrophoresis

Confirmation of ticks presumptive for tick-borne pathogens by gel electrophoresis. Rows 1-3 (from top) contain presumptive *Borrelia burgdorferi* positives, with lane 5 (from left) of row 3 representing a positive control and lane 7 of row 3 representing a negative control. Lane 1 of rows 1-3 contain a 100 bp to 1.5 kb DNA size ladder (Invitrogen). Rows 4-6 contain presumptive HGE agent positives, with lanes 25 and 26 of row 4 containing positive and negative controls respectively. Lanes 1 and 2 of rows 4-6 contain 100 bp to 12 kb and 100 bp to 1.5 kb DNA size ladders respectively. Row 7 contains presumptive *Ehrlichia chaffeensis* positives, with lanes 2 and 3 containing negative and positive controls respectively, and lane 1 contains a 100 bp to 12kb DNA size ladder. Positive reactions are indicated by a 276-bp product for *Borrelia burgdorferi*, a 334-bp product for HGE agent, and 396-bp, 486-bp, 576-bp and 666-bp products for *Ehrlichia chaffeensis*. All other reactions are negative.

APPENDIX N. Presumptive and confirmational identification of ticks collected in Santa Cruz County parks testing positive for *Borrelia burgdorferi*, the HGE agent and *Ehrlichia chaffeensis* by polymerase chain reaction.

Tick ID		Species		Sex	Location Collected	Date Collected	[DNA] ng/ul	Presumptive +			Confirmed +		
(Plate, Well)								Bb	HGE	HME	Bb	HGE	HME
P2 B1	<i>I. pacificus</i>	F	Henry Cowell SP	1/28/2000	20.50								
P2 F2	<i>I. pacificus</i>	M	Henry Cowell SP	2/24/2001	2.10								
P2 F10	<i>I. pacificus</i>	M	Henry Cowell SP	2/24/2001	2.40								
P2 G11	<i>I. pacificus</i>	F	Henry Cowell SP	3/15/2001	43.85								
P2 G12	<i>I. pacificus</i>	F	Henry Cowell SP	3/15/2001	30.55								
P2 H12	<i>D. occidentalis</i>	F	Henry Cowell SP	3/15/2001	40.15								
P3 C10	<i>I. pacificus</i>	M	Henry Cowell SP	3/15/2001	5.60								
P3 F7	<i>I. pacificus</i>	F	Henry Cowell SP	3/15/2001	29.45								
P3 H5	<i>I. pacificus</i>	M	New Brighton SB	3/11/2000	20.40								
P4 A11	<i>I. pacificus</i>	F	New Brighton SB	3/11/2000	25.35								
P4 D8	<i>I. pacificus</i>	F	New Brighton SB	4/22/2001	35.85								
P4 F9	<i>I. pacificus</i>	F	Castle Rock SP	2/6/2000	1.25								
P4 H1	<i>I. pacificus</i>	F	Castle Rock SP	2/19/2001	27.55								
P5 B2	<i>I. pacificus</i>	F	Castle Rock SP	2/17/2000	24.90								
P5 C4	<i>I. pacificus</i>	F	Castle Rock SP	2/17/2000	16.00								
P5 F6	<i>I. pacificus</i>	F	Castle Rock SP	2/19/2001	30.25								
P6 A8	<i>I. pacificus</i>	M	Castle Rock SP	2/19/2001	8.55								
P6 C9	<i>I. pacificus</i>	F	Castle Rock SP	3/24/2001	18.80								
P6 D5	<i>I. pacificus</i>	F	Castle Rock SP	3/24/2001	31.15								
P6 F11	<i>I. pacificus</i>	M	Castle Rock SP	3/24/2001	12.40								
P8 A5	<i>I. pacificus</i>	F	Big Basin SP	4/17/2001	20.10								
P8 E2	<i>I. pacificus</i>	F	Big Basin SP	4/22/2001	21.25								
P8 H5	<i>I. pacificus</i>	F	Big Basin SP	4/2/2000	28.20								
P8 D1	<i>I. pacificus</i>	F	Big Basin SP	4/8/2000	41.80								
P8 D6	<i>I. pacificus</i>	M	Big Basin SP	4/8/2000	8.35								
P9 H11	<i>I. pacificus</i>	F	Delaveaga CP	2/4/2001	17.35								
P10 E11	<i>I. pacificus</i>	F	Delaveaga CP	3/8/2001	26.40								
P10 F9	<i>I. pacificus</i>	M	Delaveaga CP	3/8/2001	5.60								
P11 A2	<i>D. variabilis</i>	M	Wilder Ranch SP	3/18/2000	18.00								
P11 A4	<i>D. variabilis</i>	M	Wilder Ranch SP	3/18/2000	27.05								
P11 B7	<i>I. pacificus</i>	M	Wilder Ranch SP	3/18/2000	6.85								
P11 C1	<i>I. pacificus</i>	M	Wilder Ranch SP	3/18/2000	7.80								

APPENDIX N continued.

Tick ID	Species	Sex	Location Collected	Date Collected	[DNA] ng/μl	Presumptive +			Confirmed +		
(Plate, Well)						Bb	HGE	HME	Bb	HGE	HME
P11 H10	<i>I. pacificus</i>	M	Wilder Ranch SP	4/12/2001	6.50						
P12 C1	<i>D. occidentalis</i>	F	Wilder Ranch SP	4/12/2001	21.00						
P12 C12	<i>D. occidentalis</i>	M	Castle Rock SP	2/19/2001	21.15						
P12 H4	<i>D. variabilis</i>	F	Castle Rock SP	2/19/2001	19.50						
P13 A5	<i>I. pacificus</i>	F	Forest of Nisene Marks SP	5/10/2001	30.95						
P13 B5	<i>I. pacificus</i>	M	Forest of Nisene Marks SP	5/10/2001	3.30						
P13 C1	<i>I. pacificus</i>	F	Forest of Nisene Marks SP	5/24/2001	19.60						
P13 C8	<i>I. pacificus</i>	M	Forest of Nisene Marks SP	5/24/2001	13.35						
P2 B6	<i>I. pacificus</i>	M	Henry Cowell SP	1/29/2000	7.80						
P2 C1	<i>I. pacificus</i>	M	Henry Cowell SP	1/29/2000	5.30						
P2 C5	<i>I. pacificus</i>	M	Henry Cowell SP	1/29/2000	10.75						
P2 E3	<i>I. pacificus</i>	F	Henry Cowell SP	2/24/2001	16.50						
P2 E6	<i>I. pacificus</i>	F	Henry Cowell SP	2/24/2001	19.70						
P2 E11	<i>I. pacificus</i>	M	Henry Cowell SP	2/24/2001	3.45						
P2 F1	<i>I. pacificus</i>	M	Henry Cowell SP	2/24/2001	3.00						
P3 A11	<i>D. occidentalis</i>	M	Henry Cowell SP	3/15/2001	14.55						
P3 D1	<i>I. pacificus</i>	M	Henry Cowell SP	3/15/2001	5.70						
P3 D2	<i>I. pacificus</i>	M	Henry Cowell SP	3/15/2001	7.55						
P3 D3	<i>I. pacificus</i>	M	Henry Cowell SP	3/15/2001	6.15						
P3 E1	<i>I. pacificus</i>	F	Henry Cowell SP	3/15/2001	15.00						
P3 F11	<i>I. pacificus</i>	F	New Brighton SB	3/4/2000	33.85						
P3 H4	<i>I. pacificus</i>	M	New Brighton SB	3/11/2000	13.30						
P4 A2	<i>I. pacificus</i>	M	New Brighton SB	3/11/2000	5.50						
P4 A4	<i>I. pacificus</i>	M	New Brighton SB	3/11/2000	8.00						
P4 A6	<i>I. pacificus</i>	M	New Brighton SB	3/11/2000	7.45						
P4 A7	<i>I. pacificus</i>	M	New Brighton SB	3/11/2000	16.45						
P4 A8	<i>I. pacificus</i>	M	New Brighton SB	3/11/2000	8.40						
P4 A10	<i>I. pacificus</i>	M	New Brighton SB	3/11/2000	5.50						
P4 B1	<i>I. pacificus</i>	F	New Brighton SB	3/11/2000	24.40						
P4 B2	<i>I. pacificus</i>	F	New Brighton SB	3/11/2000	36.15						
P4 D7	<i>I. pacificus</i>	F	New Brighton SB	4/22/2001	23.15						
P4 F1	<i>I. pacificus</i>	M	New Brighton SB	4/22/2001	5.30						

APPENDIX N continued.

Tick ID (Platelet, Weller)	Species	Sex	Location Collected	Date Collected	LDNA ng/ul	Presumptive +			Confirmed +		
						Bb	HGE	HME	Bb	HGE	HME
P5 A6	<i>I. pacificus</i>	M	Castle Rock SP	2/19/2001	1.80	no		no	no		no
P5 B9	<i>I. pacificus</i>	F	Castle Rock SP	2/17/2000	25.60	no		no	no		no
P5 E12	<i>I. pacificus</i>	M	Castle Rock SP	2/19/2001	15.00	no		no	no		no
P5 G2	<i>I. pacificus</i>	F	Castle Rock SP	2/19/2001	14.90	no		no	no		no
P5 H5	<i>I. pacificus</i>	M	Castle Rock SP	2/19/2001	8.20	no		no	no		no
P5 H9	<i>I. pacificus</i>	M	Castle Rock SP	2/19/2001	9.60	no		no	no		no
P5 H11	<i>I. pacificus</i>	M	Castle Rock SP	2/19/2001	7.35	no		no	no		no
P6 E7	<i>I. pacificus</i>	M	Castle Rock SP	3/24/2001	8.15	no		no	no		no
P7 A5	<i>I. pacificus</i>	M	Castle Rock SP	3/24/2001	3.95	no		no	no		no
P7 A11	<i>I. pacificus</i>	F	Castle Rock SP	3/24/2001	19.15	no		no	no		no
P7 B2	<i>I. pacificus</i>	M	Castle Rock SP	3/24/2001	33.90	no		no	no		no
P7 G12	<i>D. occidentalis</i>	F	Castle Rock SP	5/29/2001	20.90	no		no	no		no
P8 C12	<i>I. pacificus</i>	M	Big Basin SP	4/22/2001	6.30	no		no	no		no
P8 D1	<i>I. pacificus</i>	M	Big Basin SP	4/22/2001	6.65	no		no	no		no
P8 H10	<i>D. occidentalis</i>	F	Big Basin SP	4/2/2000	26.15	no		no	no		no
P9 B9	<i>D. occidentalis</i>	M	Big Basin SP	4/9/2000	15.85	no		no	no		no
P9 C10	<i>I. pacificus</i>	F	Big Basin SP	4/9/2000	30.70	no		no	no		no
P9 E5	<i>I. pacificus</i>	M	Sunset SB	4/10/2001	13.45	no		no	no		no
P9 H2	<i>I. pacificus</i>	F	Delaveaga CP	2/4/2001	21.65	no		no	no		no
P10 B2	<i>I. pacificus</i>	M	Delaveaga CP	2/4/2001	1.50	no		no	no		no
P10 E6	<i>I. pacificus</i>	F	Delaveaga CP	3/6/2001	19.00	no		no	no		no
P10 F1	<i>I. pacificus</i>	F	Delaveaga CP	3/6/2001	23.60	no		no	no		no
P10 H7	<i>D. variabilis</i>	F	Wilder Ranch SP	3/18/2000	48.85	no		no	no		no
P10 H10	<i>D. variabilis</i>	F	Wilder Ranch SP	3/18/2000	35.90	no		no	no		no
P11 A3	<i>D. variabilis</i>	M	Wilder Ranch SP	3/18/2000	46.75	no		no	no		no
P11 B6	<i>I. pacificus</i>	M	Wilder Ranch SP	3/18/2000	6.40	no		no	no		no
P11 G3	<i>I. pacificus</i>	F	Wilder Ranch SP	3/19/2001	40.05	no		no	no		no
P12 A12	<i>D. variabilis</i>	M	Wilder Ranch SP	4/12/2001	24.10	no		no	no		no
P12 B1	<i>D. variabilis</i>	M	Wilder Ranch SP	4/12/2001	30.20	no		no	no		no
P12 H6	<i>I. pacificus</i>	F	Castle Rock SP	2/19/2001	20.50	no		no	no		no
P13 A6	<i>I. pacificus</i>	F	Forest of Nisene Marks SP	5/10/2001	19.50	no		no	no		no
P13 C10	<i>I. pacificus</i>	M	Forest of Nisene Marks SP	5/24/2001	3.20	no		no	no		no

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