A tale of two borreliae: An epidemiological study of *Borrelia burgdorferi* sensu stricto and *Borrelia miyamotoi* in California

By

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Table of Contents:

Abstract:

Human cases of Lyme disease, caused by *Borrelia burgdorferi*, are well-documented in California, with increased risk in the north coastal areas and northwestern slopes of the Sierra Nevada range. *Borrelia miyamotoi*, a more recently identified zoonotic spirochete causing a relapsing-fever type illness, has been documented as causing human disease in the eastern United States and Europe, but human cases have not been identified in California to date. The *Ixodes pacificus*, is the primary tick vector of these two zoonotic spirochetes in California. Lyme disease became a state reportable condition in 1989 and nationally notifiable in the United States in 1991. Lyme disease is the most common tick-borne disease in the US with over 30,000 cases reported annually. However, studies have shown that Lyme disease is subject to underreporting, with estimates of up to 500,000 cases annually in the US by multiple data sources. The incidence of Lyme disease in California is low, with approximately 100 confirmed cases reported annually (0.2 confirmed cases per 100,000 population). However, California's unique ecological diversity contributes to focal highly endemic areas. The goal of this dissertation is to investigate the human epidemiology of these two zoonotic borreliae in California first with a focus on Lyme disease to reduce the burden of Lyme disease surveillance through predictive modeling, then investigating physician practices and finally by assessing human exposure to both these agents through serosurveillance.

In Chapter 1, we obtained data from the California Reportable Disease Information Exchange (CalREDIE), a secure system implemented by the California Department of Public Health (CDPH) for electronic disease reporting and surveillance. Currently, the investigation of Lyme disease is time intensive. Due to a variety of reasons, including the amount of follow-up information required due to the complexity of the Lyme disease case definition, many cases are

not followed-up completely to obtain all relevant information to correctly classify a case. In high incidence states in the US for Lyme disease, an estimation sampling approach was performed where 20% of all positive laboratory results were fully investigated which yielded accurate estimates of Lyme disease case numbers. We proposed that automatically reported information, such as lab results, and demographic risk factor information augmented with tick surveillance data would provide estimates of Lyme disease incidence similar to what would be obtained through full investigations requiring intensive follow-up. We created four predictive models using logistic regression starting with a simple model with positive and specific lab data, then successively added automatically reported and easily obtainable contextual information to each model. Our predictive models estimate was validated using k-fold cross validation with constructed ROC curves. Each of the four predictive models had very low sensitivities, which demonstrated that models based on subsets of surveillance data would underestimate the incidence of Lyme disease in California.

In Chapter 2, we surveyed physicians in California to understand knowledge and practices for testing and treating Lyme disease based on the expectation that physician awareness of recommended practices could be limited in low-incidence states like California. We compared knowledge and practice scores of physicians practicing in higher-endemic counties compared to lower-endemic counties. The risk of Lyme disease varies in California and this variation in risk can impact choices about diagnostic testing and interpretation. We found that our physicians in this study deviated from IDSA national guidelines in diagnostic testing for LD when patients sought care for both symptomatic disease and asymptomatic tick bites. Our survey results demonstrated that physicians in California could benefit from targeted education to better

understand disease risk in California and to improve recognition of symptoms and appropriate use and interpretation of serologic testing.

In Chapter 3, we evaluated and compared human exposure to *B. burgdorferi* and *B. miyamotoi* over a broad geographical range in California. We assessed human exposure to *B. burgdorferi* and *B. miyamotoi* by testing 1,700 blood bank serum samples from both higher and lower Lyme disease endemic counties in California with the hypothesis that counties with higher endemicity of Lyme disease would also have a higher endemicity of *B. miyamotoi* disease because this disease shares the same tick vector. Two of the 1,700 samples had detectable antibodies against *B. miyamotoi* (0.12%, Exact 95% CI: 0.01%, 0.42%). Both samples tested positive by C6 ELISA, GlpQ ELISA and *B. miyamotoi* whole cell western blot. Eight of 1,700 samples had detectable antibodies against *B. burgdorferi* (0.47%, Exact 95% CI: 0.20, 0.93). Samples tested positive by C6 ELISA and IgG western blot for *Borrelia burgdorferi*. Given the few seropositive samples, we could not characterize the geographic concordance between *B. burgdorferi* and *B. miyamotoi*, although we confirmed that exposure to these disease agents is low in California.

Taken together, the results of this dissertation provide insights that model-based methods using limited follow-up on cases underestimated the incidence of Lyme disease in California, which is a low-incidence state. Therefore, complete individual case follow-up as required by the current case definition is necessary to gather adequate information for accurate surveillance. Accurate surveillance information is crucial for physicians in their assessment of suspected Lyme disease patients in this low-incidence state and for monitoring Lyme disease incidence geographically.

Overall, this research validated that the risk of human infection by *Borrelia burgdorferi* and *Borrelia miyamotoi* in California is low and focal.

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Above all else *soli Deo gloria.*

List of Figures:

List of Tables:

Introduction:

The genus *Borrelia* contains two major groups of organisms: those agents that cause Lyme disease and those that cause relapsing fever and relapsing fever -like illness (1). Of those *Borrelia* species transmitted by blacklegged ticks (*Ixodes* species) in the US, *Borrelia burgdorferi* sensu stricto, also called *Borreliella burgdorferi* and hereinafter referred to as *B. burgdorferi* (2) is the principle bacterial agent of Lyme disease (LD) and infection with *Borrelia miyamotoi* causes a relapsing fever -like illness, documented in Europe and the US (2-7). Both of these *Borrelia* species have been documented in the western blacklegged tick (*Ixodes pacificus*) in California with LD being a focally endemic tick-borne disease of public health concern, and *B. miyamotoi* documented from *I. pacificus* ticks with yet-to-be-documented human disease. The purpose of this review is to summarize the literature associated with these *Borrelia* species as they relate to California with a brief overview of their respective ecology, clinical symptoms, diagnosis and treatment, and a focus on what is known about the human epidemiology.

The story of LD in North America begins with the well-known description of a cluster of juvenile arthritis cases in Old Lyme Connecticut in 1977 where Dr. Willy Burgdorfer discovered the bacteria transmitted by the *Ixodes scapularis* tick that caused the disease (3, 8). Lyme disease is most common tick-borne disease in the US with over 30,000 cases reported annually. However, studies have shown that LD is subject to underreporting with estimates closer to 500,000 cases annually in the US by multiple data sources (9-12). Lyme disease in US is transmitted by the (blacklegged tick) *Ixodes scapularis* tick in the Northeast and upper Midwest and *I. pacificus* tick in the western US, including California (2-4, 8, 13). Adding to an already

complex picture of tick-borne diseases in the US, another closely related pathogen, *Borrelia mayonii,* has been identified recently as a human pathogen (14). It is 89% to 95% similar to the *B. burgdorferi* sensu lato and has been associated with all of the clinical features of LD (15, 16). *Borrelia mayonii* has been documented to date only in Upper Midwest and northeastern US vectored by the *I. scapularis* tick (15, 16). Lyme disease is the most prevalent vector-borne disease in North America with the majority of cases occurring in the Northeast and upper Midwest (13).

The first case of LD in California was described in a 32-year-old male hiker from Sonoma County in 1978 (17). This index patient had a circular erythema migrans (reddened expanding skin lesion) with a diameter of 35 cm, eight weeks after being bitten by a blacklegged tick, presumably *I. pacificus* (17). Since then, *I. pacificus* ticks, have been detected in 56 of 58 counties in the state of California, with a range of *Borrelia* species and prevalence in *I. pacificus* populations in many of these counties. Approximately 100 confirmed cases of LD human cases are reported annually in California (9). However, the story of LD in California is not straightforward, due to the ecological complexities of the tick vector (*Ixodes* species), the many reservoir hosts of this tick species, and the multiple enzootic cycles resulting from the host-vector-agent triad. Setting the stage to this story requires understanding the etiologic agent and the ecology of *Borrelia* species in California.

Etiologic agent:

Currently, *B. burgdorferi* sensu lato consist of 21 genospecies with five genospecies of clinical relevance to humans (14, 18). *Borrelia burgdorferi* sensu stricto is one of the genospecies of

human clinical significance and is the predominant etiologic agent that causes Lyme disease in the US. It is the only *Borrelia* species that causes LD in California as documented to date (18-21).

Etiologic agent, California:

Many species of *Borrelia* have been documented in ticks, rodents, and occasionally humans in California, and together, most are part of the *B. burgdorferi* sensu lato species complex. These borreliae use small vertebrate reservoir hosts and vector ticks of the genus *Ixodes*. Each *Borrelia* species therefore differs in their ecology and geographical distribution (18) (Table i.1).

Table i.1: *Borrelia* species documented from *Ixodes* species ticks in California

Species	Suspected reservoir host	Implicated *Ixodes* species tick vector	Human disease or disease potential
B. burgdorferi ss	birds rodents small mammals	*I. pacificus* *I. jellisoni* *I. spinipalpis*	Lyme disease
B. americana (genomespecies1&2)	birds, rodents	*I. pacificus*	Unknown
B. bissettiae	rodents	*I. pacificus* *I. spinipalpis*	Potentially*
B. californiensis	rodents	*I. spinipalpis* *I. pacificus* *I. jellisoni*	Unknown
B. lanei	small mammals	*I. spinipalpis* *I. pacificus*	Unknown
B. miyamotoi	birds small mammals	*I. pacificus*	BMD*

Note: Information provided in this table are from sources (6, 22-28)
BMD = *Borrelia* miyamotoi disease
Potentially – has not been isolated from humans in the US but reports of isolation of this species in humans has been documented in Europe.

Ecosystems of California and impact on Lyme ecology:

The ecosystems of California are heterogenous and are often described by dividing the state into its four different ecoregions -- areas of distinct ecological communities of plants and animals (19). California's four ecoregions are: desert (California high deserts), Mediterranean (coast of California from Monterey Bay south to the Mexico border), forested mountains (Klamath, Sierra Nevada, Eastern Cascades), and coastal forests (North of San Francisco, Redwood Forest) (29). Tick abundance, reservoir host communities and the entomologic risk (density of infected nymphal ticks) of LD are influenced by California's ecoregion heterogeneity and climate, with infected ticks and human disease mostly occurring in the northern counties of California (Mediterranean and forested mountain ecoregions) (30-33).

Lyme disease tick vectors and reservoirs in California:

Vector-borne diseases, such as Lyme disease, are often maintained in complex transmission cycles (sylvatic or enzootic transmission cycles) between the arthropod vectors and their vertebrate host (reservoir) (34). Reservoirs for LD are defined as vertebrate host species that are commonly infected, maintain *B. burgdorferi* infection for prolonged periods of time, and are infective to the vector (34). Vector competence describes the inherent ability of the arthropod vector to become infected, maintain infection, and to transmit the disease agent (34). As mentioned, the western blacklegged tick (*I. pacificus*) in the western US and California is the primary vectors of *B. burgdorferi* to humans (35-37). *Ixodes pacificus*, the western blacklegged tick has four life stages: egg, six-legged larva, eight-legged nymph, and eight-legged adult. After the eggs hatch, the ticks must have a blood meal at every stage to survive (9). The life cycle for the western blacklegged tick requires a typical of three years to complete (38). Lyme disease in

California is principally a summertime disease and compared to the northeastern and midwestern US, human cases of LD acquired in California typically begin to peak in June, corresponding to the seasonality of the western blacklegged tick nymphal tick, the primary life stage that transmits LD to humans (39). Nonetheless, LD cases can occur throughout the year in California, due to the focal microclimates that allow for persistence of the western blacklegged tick in limited areas throughout the year (39, 40). The two important life stages of the western blacklegged tick that pose risk of LD transmission to humans in California are the adult (peak during winter and early spring) and nymphal (peak during spring and early summer) ticks (35, 41). However, nymphal ticks pose the highest risk of LD transmission to humans in California, because the minimum infection prevalence (MIP) of nymphal ticks infected with *B. burgdorferi* sensu lato is higher than in adults. The MIP is defined as: [(number of positive tick pools) / (number of total ticks tested)] x 100 (42). The MIP in nymphal ticks range from 0.9% to 50% with an average of 3.2% (compared to 0.3% to 10% in adults, average MIP 0.6%) (32, 35, 39, 43), and they are difficult to detect when attached and which delays removal.

Enzootic cycles:

A comparison of enzootic cycles of *B. burgdorferi* in the eastern US to the enzootic cycles in California is illustrative to understand why the risk of LD in humans is so different in California. While LD is endemic in both the eastern and western US, the pathogen is maintained by different vector species in distinct enzootic life cycles comprised of different reservoir hosts (44, 45). In the eastern US, the enzootic transmission cycle is maintained principally by the single species vector, the deer tick or simply blacklegged tick (*I. scapularis*) and a single mammalian host, the white-footed mouse (*Peromyscus leucopus*) (46-48) which results in a high prevalence of

nymphal (range 20% to 52%) (49) and adult (mean 49% (range 17% to 79%) ticks infected with *B. burgdorferi* (50).

In contrast, the many habitats of California results in varied enzootic cycles for *B. burgdorferi*. The enzootic transmission cycles in California are maintained separately by at least three *Ixodes* species *I. spinipalpis, I. jellisoni, and I. pacificus,* with their three respective mammalian hosts, dusky-footed woodrat (*Neotoma fuscipes*), kangaroo rat (*Dipodomys californicus*), and the western gray squirrel (*Sciurus griseus*) (20, 21, 34, 51, 52). In all these enzootic transmission cycles, the western blacklegged tick, appears to be an inefficient maintenance vector (vector that keeps *B. burgdorferi* cycling in nature) (34), however, it serves as a bridge vector (vector that transmit *B. burgdorferi* to humans) (34) from rodent host to human because *I. spinipalpis* seldom bite humans and *I. jellisoni* are not known to bite humans at all (51). Inefficient host-to-tick transmission could be a factor that results in low prevalence of *B. burgdorferi* in *I. pacificus* ticks (0% to 20% infection prevalence) (32, 43) compared to the prevalence of *B. burgdorferi* in *I. scapularis* ticks (27% to 45% infection prevalence) (53, 54) elsewhere in the US (34, 45). Another important western blacklegged tick host is the western fence lizard (*Sceloporus occidentalis*) and southern alligator lizard (*Elgaria multicarinata*) (55). These lizard hosts serve as the major source of a blood meal for larval and nymphal *I. pacificus* ticks, but they are an incompetent reservoir for *B. burgdorferi* because a complement-related substance in the blood of these lizards actively kills *B. burgdorferi* during tick feeding (56, 57). Not intuitively, the presence of western fence lizards, even though an incompetent reservoir for the *Borrelia* pathogen, may in fact increase disease risk to humans. By existing as the principal blood meal host to immature ticks, lizards facilitate the survival of the larval and nymphal tick to the next

life stage when it can take a blood meal on an infected small mammal, thus resulting in a higher density of infected ticks compared to areas with fewer lizards (55).

Influence of climate and weather:

The life cycles of the blacklegged ticks are sensitive to changes in climate and weather conditions (58). The effects of climate change on tick-borne diseases can alter the magnitude and geographic distribution of tick vector (40). For example, the geographic range of LD in the US has expanded, particularly in the northeast where the number of counties at high risk for LD has increased by more than 320%, from 43 counties in the mid 1990's to 182 counties in 2012, where most of the expansion occurred in the northern regions of the northeast US (59). However, unlike the deer tick, the western blacklegged tick has expanded very little eastward since the last survey performed in the 1990s (60). However, the *I. pacificus* nymphal ticks have been found in oak woodland habitats at higher elevations up to approximately 1,500 m, in the Sierra Nevada foothills driven partially by canopy cover (61). The western blacklegged tick remains mainly established along the Pacific coastal regions and moist foothills of the Pacific states (Washington, Oregon, and California), but also occurs in the more arid inland states of Arizona, Nevada, and Utah with moist micro-habitats (62). There are several explanations for the relatively stable western blacklegged tick distributions in the western US: the tick has reached its fundamental niche (63), there are substantial barriers to migration such as mountains ranges and vast deserts (63), or competition between the established *I. scapularis* populations may have prevented the western blacklegged tick from moving outside the west (63). Overall, climate change has affected the distribution of *I. scapularis* tick but has not appeared to affect the distribution of the *I. pacificus* tick.

Clinical presentation of Lyme disease:

Lyme disease can cause varied clinical manifestations which divided into early localized (3 to 30 days after exposure) and late disseminated (days to months after exposure) signs and symptoms to facilitate the diagnosis of Lyme disease (8, 64, 65). Early localized LD is characterized by an erythema migrans (EM) rash which occurs in about 70% to 80% of cases (9, 64). An EM is described as an expanding erythematous rash typically larger than five cm, often with a well-demarcated outer border and central clearing ("Bull's eye" rash); however, some EM rashes may be more diffuse with no blanching (64). Early disseminated disease can present over days to weeks post-exposure, where patients can develop multiple EM rashes, acute neuroborreliosis (eg, meningitis, facial palsy, or radiculopathy), or Lyme carditis- a condition which can be fatal (66-68). Late disseminated disease is normally characterized as Lyme arthritis, which involves severe joint pain and intermittent swelling typically of large joints, particularly the knees (9, 64, 69). After months to years, untreated Lyme disease may develop into late neuroborreliosis (eg, Lyme encephalopathy, radiculoneuropathy, or paresthesias) (8, 70-72).

Clinical Lyme disease in California:

Molecular analysis of *Borrelia burgdorferi* spirochetes infecting humans is a powerful way to improve the understanding of LD ecology, and epidemiology (73). Genotypic analysis has been useful to identify exposure and are vital for diagnostic test development and validation (74). Genotyped strains of *B. burgdorferi* have been isolated from skin, blood/serum, and synovial fluid (73, 75, 76). However, there are very few genotypic characterizations from human samples in the western US. For example, *B. burgdorferi* was isolated from skin biopsy samples of three patients in California in whom LD was diagnosed (75). *Borrelia burgdorferi* was also isolated

from human sera through PCR testing in 22 patients from Northern California (73). *Borrelia burgdorferi* was also identified from synovial fluid of a 12-year-old male through PCR (76).

Diagnostic testing/treatment:

The clinical diagnosis of LD is based upon medical awareness of potential exposure to infected ticks, clinical manifestations, and laboratory results (9, 77, 78). Currently, serologic testing is the principal means of laboratory diagnosis of LD (79). The recommended laboratory serologic testing by the Infectious Disease Society of America (IDSA) supports a two-tiered approach. The first tier is typically an enzyme immunoassay screen (EIA), immunofluorescence assay (IFA) or enzyme-linked immunosorbent assay (ELISA) antibody test, which measures overall antibody response (typically IgM and IgG) to the antigen *B. burgdorferi* (80). The second tier should be performed only if the first-tier test is either positive or equivocal. The second-tier test is a confirmatory western blot test, either IgM within first 30 days post onset or IgG thereafter (80). The second tier immunoblot is a serologic test that detects antibodies against a set of preselected protein antigens produced by *B. burgdorferi* (81). The two-tiered testing approach when performed in accordance with the IDSA testing guidelines is both highly sensitive (70% - 100%) and specific (> 95%) in diagnosing disseminated LD (69). The IDSA guidelines outlines complete testing strategy for LD (80).

On July 29, 2019, the Food and Drug Administration (FDA) approved the Zeus Scientific serologic assays for the diagnosis of LD. The Zeus serologic assays replace the second tier immunoblot with a second ZEUS ELISA referred to as a modified two-tier methodology approach in the diagnosis of LD (82).

Diagnosing Lyme disease in California:

Effective diagnostic approaches depend on a health care provider's knowledge and awareness of LD in their community of practice (83). While many diagnostic tests perform well, none are perfect. When determining whether to test for LD, physicians must consider a patient's pretest probability which is directly affected by disease prevalence (84). Pretest probability is defined as the probability of a person having LD before testing. The pretest probability becomes crucial in the diagnosis of LD in a low incidence state such as California and should be based on the probability of tick exposure and clinical findings (85). In a low incidence state for LD, the pretest probability is low, increasing the false positive rate. Indeed, in a low incidence state like California, the false positive rate may exceed than the true positive rate (69, 75). The patient's travel history and clinical exam should be part of the equation in determining the patient's pretest probability, especially in a low-incidence state. The challenge for physicians in diagnosing LD in a low incidence state requires determining if testing is appropriate. Testing is appropriate when [1] a patient has symptoms that could yield a positive result if they are infected (i.e., should be some possibility of disseminated disease and exposure) and [2] three to four weeks have elapsed since infection to allow for a detectable antibody response to develop (71, 86).

Treatment of Lyme disease:

Lyme disease is treatable with the correct antibiotic treatment. Treatment for LD depends on the stage at which it is diagnosed. The standard treatment for early-stage LD is oral antibiotics typically those in the tetracycline or cephalosporins classes (80). Doxycycline is typically used in adults and children older 8 years of age, or amoxicillin or cefuroxime for adults who cannot tolerate doxycycline, younger children, and pregnant or breast-feeding women for 14 to 21 days

(80). When LD progresses untreated, it may involve the central nervous system, in which case a 14-to-28-day course of intravenous antibiotics is usually recommended (80). The majority of patients diagnosed with early LD recover without further complications following treatment with antibiotics. However, approximately 10% of patients develop post-treatment Lyme disease syndrome (PTLDS) (87). These patients experience persistent symptoms for six months or greater which negatively impacts daily functioning. Studies demonstrate that retreatment of antibiotics provides no benefit and carries significant risk to the patient (88, 89).
Antibiotic prophylaxis (a single oral dose (200mg) of doxycycline) may be administered within 72 hours after removal of the tick in high incidence states (northeastern to upper midwestern US) and when the tick has been attached longer than 36 hours. (90-93). However, antibiotic prophylaxis is not recommended in low incidence states such as California because the risk of infection from a tick is low compared to the risk of a negative antibiotic reaction (75).

Lyme disease risk is geographically clustered, with the highest risk being in the northeastern and upper midwestern part of the US (94). With the increasing number of cases and the geographic spread of LD coupled with the fact that individuals can get LD more than once when bitten by an infected tick, compels and complicates the development of novel effective vaccines to control this vector-borne illness (94). Two vaccines were developed back in the 1990's and were based on the outer surface protein A (OspA) of *B. burgdorferi* (95). Although this vaccine was effective, the use of the vaccine in the general population was low and it was eventually discontinued by the manufacturer in 2002 (96). However, a second-generation OspA vaccine containing 6 different serotypes entered a phase two clinical trial in 2017 (94).

Epidemiology and surveillance of Lyme disease:

Lyme disease is the most common tick-borne disease reported in the USA, with an estimated 30,000 cases reported annually. However, LD is subject to underreporting and through the use of multiple data sources it is estimated that the actual number of cases are upwards of 500,000 cases annually in the US (9-11). The highest incidence of LD is in the northeastern and mid-Atlantic US. This geographic area accounts for over 90% of all reported confirmed cases (13). Surveillance for LD entails further investigation to both collect clinical and exposure history for both high incidence states as well as low incidence states (97). Lyme disease became a nationally notifiable disease in the US in 1991 (9). In California, health care providers, hospitals and laboratories report cases of LD to state or local health jurisdictions, who then in turn categorize the reports into case classifications according to a standardized surveillance case definition developed by the Council State and Territorial Epidemiologists (CSTE) (98). A surveillance case definition is a set of uniform criteria used to define a disease for public health surveillance. Surveillance case definitions enable reporting jurisdictions to classify and count cases consistently. Surveillance case definitions are not intended for clinical diagnosis or determining treatment for an individual patient (98). For surveillance purposes, the current posted CDC (2017) case definition describes a confirmed case of LD as any patient with a physician diagnosed EM rash at least 5cm in diameter and/or two-tiered serologic testing with compatible clinical illness with exposure being less than or equal to 30 days before onset of symptoms and to an area of potential tick habitats (98). Surveillance data are acquired by county of residence, not county of exposure. Consequently, case reports from a particular state are not evidence of local transmission (13).

Epidemiology and surveillance of Lyme disease in California:

Lyme disease has been a reportable disease in California since 1989 (99). California has a low incidence of LD with approximately 100 confirmed cases reported annually (0.2 confirmed cases per 100,000 population) (CDPH, 2005-2010). However, California's unique ecological diversity contributes to focal high endemic regions (30, 100-102) In addition, approximately one-third of cases reported in California are exposed from the upper Midwest to northeastern US (103). The confirmed cases for LD in California are mostly male with a bimodal age distribution between 10 to14 years and over 50 years (103). These demographic characteristics are similar to what is seen in the US with the highest rates of LD among males between the ages of 5 to 15 years and over 50 years (13). Case rates among males are slightly greater than females (13).

In California, LD is electronically reported through the California Reportable Disease Information Exchange (CalREDIE) via healthcare providers and laboratories. California has seen a marked increase of case reports for Lyme disease due to the advent of electronic case and laboratory reporting of reportable infectious diseases in 2011. However, the incidence of cases meeting the confirmed case criteria has remained fairly stable, despite the increase in case reports. The case report increase may overburden local health departments who must investigate all reported positive lab reports to gather the necessary clinical information to classify a case of LD. High incidence states for LD, such as New York, New Jersey, and Massachusetts have used estimation sampling approaches to approximate the incidence of LD in their respective states (97, 104).

New hard tick transmitted relapsing fever causing *Borrelia* species*: Borrelia miyamotoi*:

Borrelia miyamotoi is the causative agent of *B. miyamotoi* disease, also referred to as a relapsing fever like illness since it is genotypically clustered with other relapsing *Borrelia* spp (105, 106). *Borrelia miyamotoi* is a spirochete transmitted by the same hard-bodied (*Ixodes* species) ticks that are vectors of *B. burgdorferi* and other LD agents (5, 106-110). These include *I. scapularis* in the northeastern and north-central US and adjoining areas of Canada; *I. pacificus* in the far-western US and British Columbia; *I. ricinus* in Europe, and *I. persulcatus* in Europe and Asia (42, 107, 108, 110, 111). *Borrelia miyamotoi* infections are transmitted to humans by ticks that had acquired the organism either through transstadial (infection passed between stages after molting) or transovarial transmission (infection passed from adult female to offspring) (112) (5).

Borrelia miyamotoi was first identified in 1994 from the *I. persulcatus* tick and blood of the Japanese field mouse (*Apodemus argenteus*) in Hokkaido, the northernmost island of Japan (107). Globally, the prevalence of *B. miyamotoi* in host-seeking *Ixodes* spp ticks ranges from 1 to 10% (108, 113, 114).

The reservoir hosts of *B. miyamotoi* throughout much of its distribution are poorly known or unknown. However, in the northeastern and upper midwestern US, the white-footed mouse (*P. leucopus*) and wild turkey (*Meleagris gallopavo*) appear to be competent reservoir hosts (5, 115).

Despite the presence of *B. miyamotoi* found in human biting *Ixodes* vector and its close relation to other borreliae causing tick-borne relapsing fever, the pathogenicity of *B. miyamotoi* was unclear until the first human cases were reported in Russia in 2011 (110). Since then, human

cases of *B. miyamotoi* have been documented in Europe, Japan and Eastern and midwestern USA, however, there have been no human cases of *B. miyamotoi* documented in California. (6, 110, 116).

Clinical presentation of *Borrelia miyamotoi*:

The most commonly reported clinical presentation of *B. miyamotoi* infection is a febrile illness consisting of fever that may exceed 40°C, fatigue, headache, chills, myalgia, arthralgia, and nausea (5, 6). Some patients may experience relapsing fever type illness which is characterized by two or more episodes of febrile illness lasting 1 to 5 days each with intervals of non-febrile periods lasting at least 2 to 7 (117). To date, a maximum of three febrile episodes have been documented in patients with *Borrelia miyamotoi* disease (110). General laboratory findings with *B. miyamotoi* infection are leukopenia, thrombocytopenia, and elevated aminotransferase levels (105, 116).

Diagnosis/treatment of *Borrelia miyamotoi*:

The diagnosis of *Borrelia miyamotoi* infection should be considered in any patient who resides in or has recently traveled to a region where Lyme disease is endemic and develops repeated febrile illness since the pathogens that causes LD and *B. miyamotoi* disease are vectored by the same tick (5). Unlike other tick-borne diseases transmitted by *Ixodes* ticks, it is conceivable that *B. miyamotoi* infection can be acquired by humans from the bite of a larval tick, since the agent may be transmitted transovarially in the tick (118). It has been suggested that *B. miyamotoi* infection is maintained in the salivary gland of unfed hard ticks (118, 119), suggesting that *B. miyamotoi* infection is transmitted more rapidly to humans, whereas *B. burgdorferi* is known to

require several days to transmit (≥ 36hours), due to an obligatory development phase in the tick gut when feeding commences for transmission (120-122).

Diagnosis of *B. miyamotoi* relies on consistent clinical findings such as fever, fatigue, and headache, complete travel history coupled with polymerase chain reaction (PCR) tests that detect DNA of the organism from the patient (5, 105) and/or serologic testing comprised of a two-tiered antibody assay (first step EIA, second step western blot) that detects glycerophosphodiester phosphodiesterase (GlpQ) specific antibodies during the acute and convalescent stages of infection (6, 123). The GlpQ antigen will distinguish serologically between human cases of relapsing fever and LD however, the GlpQ protein may cross react with other relapsing fever *Borrelia* spp (124)

To date there have been no therapeutic trials or comprehensive studies to evaluate treatment regimens, therefore, optimal antibiotics choice, dosage, and duration of treatment have yet to be determined for *B. miyamotoi* infection (5, 105). Treatment recommendations for *B. miyamotoi* infection are based on case reports and series that have been published thus far (6, 117, 123). Doxycycline (100 mg orally every 12 hours) given for 7 to 14 days is the most commonly prescribed antibiotic for patients experiencing uncomplicated *B. miyamotoi* infection to date (6, 7, 110, 125, 126).

Epidemiology of Borrelia miyamotoi:

Borrelia miyamotoi has been detected in all *Ixodes* spp ticks that vector LD with infection prevalence rates ranging from 0% to 10% (5). The infection prevalence for *I. persulcatus* (range

– Europe to central and northern Asia) was 3.60%: adult and nymphal ticks had similar prevalence (2.41% and 3.85% respectively) (127, 128). The infection prevalence for *I. ricinus* (range – Europe, North Africa, Russia, Middle East, and Iceland) was 1.25%; with adults *I. ricinus* at 1.53% and nymphs at 1.17% (127, 128). The infection prevalence for *I. scapularis* (range – eastern to northern Midwest US to southeastern Canada) was adults 2.00% and nymphs 1.99% (5, 129).

Epidemiology of *Borrelia miyamotoi* in California:

The prevalence of *B. burgdorferi* is typically higher in nymphal western blacklegged tick (~3-5%) than adult ticks (~1% or less) (42), while the prevalence for *B. miyamotoi* is about the same (~1%) in both tick life stages (130-134). *Borrelia miyamotoi* is transmitted both transstadially and transovarially, which means that while the risk of exposure to *B. burgdorferi* is greater than *B. miyamotoi* after exposure to nymphal ticks, *Borrelia miyamotoi* infection may occur after larval, nymphal, or adult tick exposure extending the season of risk (42). The distribution of *B. miyamotoi* in *I. pacificus* ticks appears to be similar to that of *B. burgdorferi* and is most prevalent in coastal and foothill regions of northern California (42, 135). Despite ample evidence of *B. miyamotoi* in California ticks, including ticks that were recovered from humans (136), epidemiological information and case descriptions of *B. miyamotoi* infections in humans are lacking in California.

To date, there have been no human cases of *B. miyamotoi* reported in California. The low prevalence of *B. miyamotoi* in ticks across different habitats even in higher endemic areas in California for Lyme disease (42) suggests that the host species may not be a major factor

influencing the prevalence of infection in ticks (115, 129, 137, 138). It has been postulated that in western US including California, there may not be strong amplifying reservoir hosts for *B. miyamotoi*, and that enzootic transmission involving most, or all hosts, is inefficient (139). Moreover, since *B. miyamotoi* is maintained in ticks via transovarial (from female to egg) as well as transstadial (between tick life stages) transmission, a vertebrate reservoir host may not be necessary to maintain the infection (108, 113, 114). Also, the lack of genotypic diversity among *B. miyamotoi* suggests that transovarial transmission may play a more significant role in the perpetuation of *B. miyamotoi* (139).

Goals of this dissertation:

The goal of this dissertation is to evaluate the current understanding of hard tick associated *Borrelia* infections in California, specifically *B. burgdorferi* and *B. miyamotoi*. Chapter 1 of my dissertation will address the complexity of collecting information for case classification for LD in California. The human epidemiology of LD in California is largely understood from the public health surveillance data, currently collected electronically through the California Reportable Disease Information Exchange (CalREDIE). Local health jurisdictions are tasked to follow up with all case reports, however, due to a variety of reasons, many case reports are not followed up to properly classify a case as Confirmed or Probable (both reportable outcomes). We evaluated subsections of the CalREDIE data from 2011 to 2017, in combination with available county-level tick positivity data from the state, to evaluate whether reduced subsets of information, requiring little to no follow-up by local health jurisdictions, might adequately capture reportable cases to inform the understanding of human epidemiology of LD in California. This evaluation proposes to simplify data collection necessary for case classification to reduce the burden on

local health jurisdictions without compromising specificity of LD case inclusion, thereby creating a more comprehensive understanding of LD in California.

Chapter 2 will address California physician knowledge and practice of the testing and treatment of Lyme disease- an understudied area in this low incidence state. We conducted a survey of physicians in California to determine if the knowledge and practice of testing and treating Lyme disease differed among physicians practicing in higher endemic counties compared to lower endemic counties. The results could help inform physicians and public health of subject areas to focus education to improve the testing and treatment of LD in a low incidence state.

Human infection with *B. miyamotoi* in California residents remains under-studied. Chapter 3 focuses on distribution of human exposure to *B. burgdorferi* and *B. miyamotoi* in a large blood bank sample to estimate both prevalence and geographic risk of exposure to these agents. Findings from this study should inform public-health messaging for Californian's overall risk to *B. burgdorferi* and *B. miyamotoi*.

Taken together, the retrospective surveillance system analysis, physician knowledge attitude and awareness assessment, and serologic survey of both the human infecting *Borrelia* species will inform approaches to improve the understanding of the epidemiology of *B. burgdorferi and B. miyamotoi* in Californians.

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Chapter 1: Lyme disease surveillance in California: Performance of predictive modeling for disease classification

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Abstract:

Lyme disease is the most common reported vector-borne disease in United States. Lyme disease has been a reportable disease in California since 1989 and became nationally notifiable in the United States in 1991. The purpose of public health surveillance of Lyme disease is to assess changes in incidence, monitor for geographic expansion of Lyme disease and to inform public health educational materials to prevent disease.

California surveillance data on Lyme disease is currently collected primarily through electronic laboratory reporting through the California Reportable Disease Information Exchange (CalREDIE). Reporting of cases begins at the local (typically county) level where case reports are evaluated and classified following a national standard case definition; cases are then forwarded to the state for review and closure. Cases classified as confirmed and probable are forwarded to the Centers for Disease Control and Prevention National Surveillance system. Due to a variety of reasons, including the amount of effort needed to gather the necessary follow up information given the complexity of the Lyme disease case definition, many cases lack all relevant information necessary to classify a case.

The purpose of this research is to assess if modeling approaches using automatically reported data from CalREDIE plus tick surveillance data would be enough information to correctly classify a case or if further follow up necessary, such as clinical or travel information. We created four predictive models using logistic regression. K-fold cross validation (10-fold) was used to estimate how accurately the predictive models performed in practice. Our results showed modeling approaches using automatically reported data, tick surveillance data and follow-up travel or clinical information were not sufficient alone and that full Lyme disease investigations were necessary to improve the overall sensitivity and specificity of California's surveillance data. We anticipate that the results from this research can inform local and state health department in low incidence states, such as the Californian Department of Public Health, on strategies to enhance surveillance practices for Lyme disease.

Introduction:

Public health or epidemiologic surveillance is defined as the ongoing systematic collection, analysis and interpretation of health data essential to the planning, implementation, dissemination, and to facilitate the prevention and control of disease (1, 2). The purpose of surveillance is to assess local changes in incidence, monitor for geographic expansion of Lyme disease and to drive educational materials aimed at providers and the public.

Surveillance case definitions enable public health officials to classify and count cases consistently across state and local reporting jurisdictions (2). Surveillance case definitions are not intended to be used by healthcare providers for making a clinical diagnosis (2). All national notifiable conditions are reported to California Department of Public Health (CDPH) via local health departments (LHD) by a set of uniform criteria called a surveillance case definition, established by the Council of State and Territorial Epidemiologists (CSTE), which involve a combination of laboratory support, clinical evidence, and additional risk factors such as travel to endemic areas within the incubation period (2). California State law mandates that Lyme disease and other specified diseases and conditions be reported by healthcare providers and laboratories to the public health authorities. The California Reportable Disease Information Exchange (CalREDIE) is a secure system that the California Department of Public Health (CDPH) has implemented for electronic disease reporting and surveillance. The CalREDIE surveillance system was further improved with the advent of electronic laboratory reporting in 2011.

Lyme disease, caused by a tick-borne bacterium, *Borrelia burgdorferi*, is reportable at the national and state level. It is the most commonly reported vector-borne disease in the United

States (3-5). Lyme disease has been a reportable disease in California since 1989 (6) and became a nationally notifiable disease in the United States in 1991 (3). The surveillance case definition for Lyme disease has changed multiple times since Lyme disease became a national notifiable condition, reflecting both the improved diagnostic testing and understanding of the epidemiology of Lyme disease in the US. The 2017 surveillance case definition was the last update (7).

The western blacklegged tick, *Ixodes pacificus,* is the species of tick in California that transmits *B. burgdorferi* to people (8). In California, the Vector-Borne Disease Section (VBDS)- CDPH and other local vector control agencies, collect ticks and test them for tick-borne disease-causing agents as part of environmental surveillance. Tick surveillance is intended to generate estimates of local prevalence of specific pathogens in nymphal and adult life stages of the *I, pacificus* tick (9). Tick surveillance provides information as to when and where humans are at risk for exposure to ticks and tickborne pathogens (9). The western blacklegged tick has been found in 56 of the 58 counties in California (10). The western blacklegged tick is commonly found in the humid northern California coastal areas and along the western slope of the Sierra Nevada range (10-12). In general, risk of adult *I. pacificus* bites to humans occurs from the fall until spring (October until May), with a peak in January (13). The risk of nymphal *I. pacificus* is highest in spring (April–June) but can occur anytime from January until October (13).

For the past 20 years, the incidence of Lyme disease has remained fairly stable at approximately 0.2 confirmed cases per hundred thousand (3, 14), despite a marked increase in Lyme disease reporting due to the advent of electronic case and laboratory reporting of reportable infectious

diseases in 2011 (14). An increased number of incomplete Lyme disease case reports that require follow-up may overburden local health departments who must investigate all reported positive lab reports to gather the necessary clinical information to classify a case of Lyme disease. Due to a variety of reasons, including the amount of effort needed to gather the necessary follow up information given the complexity of the Lyme disease case definition, many cases lack all relevant information necessary to classify a case. One approach that states with a high incidence of Lyme disease ($\geq$ 10 cases per 100,000), such as New York, New Jersey, and Massachusetts have used is an estimation sampling approach to approximate the incidence of Lyme disease in their respective states (15, 16), since the high number of case reports precludes extenstive follow up efforts. Estimation sampling procedures have shown to be a good approximation of the incidence of Lyme disease in their high incidence states (15, 16).

The purpose of our research was to assess if estimation procedures through modeling could approximate the incidence of Lyme disease in a low incidence state, such as California, which would decrease the need for extensive case follow up in face of the increased number of laboratory reports. In this research, we used predictive modeling to assess if automatically reported data from CalREDIE, plus readily available tick surveillance data, would provide adequate information to correctly estimate case status. Surveillance data obtained from CalREDIE was the standard in which we used to compare our predicted models against, as it is the most complete source of data estimating Lyme disease incidence in California.

Methods:

Human surveillance data:

Surveillance data on Lyme disease were acquired from the California Department of Public Health (CDPH) Surveillance Statistics Section from the State of California electronic reporting system (CalREDIE) database covering the time period January 1, 2011, through December 31, 2017. The data obtained from the Surveillance Statistic Section of the California Department of Public Health were de-identified of all major personal identifiers.

The inclusion criteria included complete demographic data for variables used in the models such as age and sex, A California resident classified in one of the four levels of classification (confirmed, probable, suspect, not a case) (17, 18). All duplicate records were excluded in the final analysis. The following were the variables used in the predictive models. All positive laboratory results for Lyme disease are automatically reported to CalREDIE electronically. The variable IgG western blot was included in the analysis as it can be used as a single tier test in the surveillance case definition (2011, 2017 case definitions) and was more reliably collected than the IgM western blot. The demographic variables used were sex and age as they are predictors of Lyme disease (19). These variables are automatically reported to CalREDIE along with the positive lab data. The variable episode date in CalREDIE represents the earliest date associated with the case record and typically related to the date of onset of disease. Therefore, we used episode date as a proxy to represent season which is also a predictor of Lyme disease (19). Both clinical and travel information were also included in the models, but these variables require further follow-up with either the provider or the patient. The clinical variables included were presence of a physician diagnosed erythema migrans (EM) > 5cm or presence of at least one of

the disseminated symptoms listed in the Lyme disease surveillance case definition (7). The travel variable included was from patient interviews regarding their possible exposure area.

Adult tick surveillance data:

Data on adult *I. pacificus* ticks collected and tested as part of the VBDS-CDPH environmental surveillance program were gathered from VBDS annual reports published each year by CDPH covering the years of 2000 – 2019 (14, 20-23). The data fields collected were county where ticks were collected, number of ticks or tick pools collected and number of positive ticks or positive tick pools for *B. burgdorferi* sensu lato. The minimum infection prevalence (MIP) was calculated for adult ticks. The MIP is defined as [(number of positive tick pools) / (number of total ticks tested)] x 100 (11). A tick pool is positive when at least one tick in the pool is positive (11). CalREDIE data include county of residence for each case report. Therefore, county level tick data were merged with CalREDIE surveillance data by county so that each case record was associated with the tick MIP for that county.

Predictive modeling:

The dependent (outcome) variable was categorized as a binary variable: reportable (confirmed, probable) and not reportable (suspect, not a case). Four different predictive models were created using logistic regression, each adding additional information that is either automatically reported in the CalREDIE system, available through the state, or that would require further investigation from the county (as is currently expected to be done in CalREDIE). Model 1 included lab data only - specifically IgG western blot. Model 2 included IgG western blot results, demographic variables and adult tick data. Model 3 included an IgG western blot, demographic variables,

adult tick data and clinical information which requires further follow-up. Model 4 included an IgG western blot, demographic variables, adult tick data and travel history which requires further follow-up (Table 1.1).

K-fold cross validation (10-fold) was used to estimate the performance of these predictive models. The CalREDIE data were randomly partitioned into approximately 10 equal groups (folds). Through the 10-fold cross validation, each model was fit on a training set (comprised of 90% of the data) and then evaluated on a test set (remaining 10% of the data) 10 times, so that each fold acts as a test set at some point.

For each predictive model, sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), accuracy and Area Under the Curve (AUC) were calculated for each test set and then averaged across the 10 test sets for an overall estimate. Sensitivity was defined as the proportion of true positives (reportable cases) that were correctly identified by each model (24) while specificity was defined as the proportion of true negatives (non-reportable cases) that were correctly identified by each model (24). The positive predictive value (PPV) was defined as the proportion of those predicted reportable by the models who were reportable by CalREDIE. Negative predictive value (NPV) was defined as the proportion of those predicted not reportable by the models who were not reportable by CalREDIE (25). Accuracy was defined as the model's ability to differentiate between a reportable and not reportable cases (26). A Receiver Operating Characteristic (ROC) curve was created for each of the 10 test sets and will be reported. The ROC curve was created by plotting the true positive rate (sensitivity) against the false positive rate (1-Specificity) (27). The area under the receiver operating characteristic curve or AUC, is a

performance metric that evaluated the four predictive models and was also computed (27). The AUC provided an aggregate measure of performance across all possible classification thresholds for a model. An AUC of 1 represents a perfect predictive model and an AUC of 0.5 represents a predictive model performing no better than chance (26, 27). Each of the four predictive models produced 10 ROC curves (one for each test set), each with a corresponding AUC. An average AUC was calculated and reported.

Ethics approval:

De-identified surveillance data for Lyme disease were analyzed for this study. The study was determined to be exempt for human subjects review by the Institutional Review Board, University of California Davis (Protocol Number 1090480-1_revised11062017, FWA No: 00004557) and by the California Health and Human Services Agency's Federal wide assurance #00000681, Project number: 17-06-3028.

Table 1.1: Variables included in each predictive model

Variables	Model 1 (lab)	Model 2 (Lab, Dem, Tick)	Model 3 (Lab, Dem, Tick, Clin)	Model 4 (Lab, Dem, Tick, Trav)	Explanation of variable
IgG Western Blot	**X**	**X**	**X**	**X**	Categorical, positive, negative, no IgG performed
Demographics					
Sex		**X**	**X**	**X**	Categorical, male, female
Age		**X**	**X**	**X**	Categorical, six age categories ~ 15-year intervals
Adult tick data		**X**	**X**	**X**	Continuous variable, calculated MIP per county
Season		**X**	**X**	**X**	Categorical, fall, winter, spring, summer
Clinical *			**X**		Binary for EM >5cm and binary for disseminated symptoms
Travel *				**X**	Categorical, high incidence, low incidence, unknown/no travel

Adult tick data calculated MIP = (Number of positive tick pools / (number of total ticks tested) x 100
* Variables that are not automatically reported in CalREDIE

Results:

Between the time period of January 1, 2011, to December 31, 2017, there were over 13,000 positive lab reports submitted to CalREDIE for Lyme disease. After applying our inclusion criteria and eliminating all duplicate records, we ended up with 11,914 lab reports that were included for our final analysis, including 787 (7%) records that were reportable (confirmed or probable) and 11,127 (93%) records that were non-reportable (suspect or not a case) cases. Of the 11,127 non-reportable records, 9,064 (81%) were classified as not a case and 9,260 (83%) were reported to CalREDIE via electronic laboratory reporting.

Of reportable cases, over 50% were male. Of not reportable cases over 60% were female. Age was categorized into six age categories with the two highest percentage of reportable cases in the 0-year-old to 14-year-old age group (19.6%) and 45-year-old to 59-year-old age group (22.4%). More reportable cases were reported during the summer months (48.8%) and fewer were reported during the winter months (11.8%). About one-third of reportable cases in California were attributed to travel to a high-incidence state. About 50% of the reportable cases had a positive IgG western blot while over 70% of the non-reportable cases had no IgG western blot performed or a negative result. Thirty-one percent of reportable cases had a physician-diagnosed EM greater than 5cm and over 50% of reportable cases had at least one disseminated symptom. All predictive demographic variables were statistically different between our reportable and non-reportable cases (Table 1.2).

Table 1.2: Demographics of reportable and not reportable Lyme reports			
Variables included in model	**Reportable (N=787)**	**Not Reportable (N=11127)**	***P*-Value**
Sex*			<.0001
Male	415 (52.7)	3620 (32.5)	
Female	372 (47.3)	7507 (67.5)	
Age*			<.0001
0 – 14 years	154 (19.6)	937 (8.4)	
15 – 29 years	130 (16.5)	2167 (19.5)	
30 – 44 years	152 (19.3)	2811 (25.3)	
45 – 59 years	176 (22.4)	3049 (27.4)	
60 – 74 years	151 (19.2)	1733 (15.6)	
75 +	24 (3.0)	430 (3.9)	
Season*			<.0001
Fall	154 (19.6)	2895 (26.0)	
Spring	156 (19.8)	2635 (23.7)	
Summer	384 (48.8)	3312 (29.8)	
Winter	93 (11.8)	2285 (20.5)	
Travel*			<.0001
**High Incidence	237 (30.1)	88 (0.8)	
***Low Incidence	348 (44.2)	468 (4.2)	
Unknown/None	202 (25.7)	10571 (95.0)	
Positive IgG WB*			<.0001
Positive	394 (50.1)	295 (2.6)	
Negative	163 (20.7)	2264 (20.4)	
No serology	230 (29.2)	8568 (77.0)	
EM > 5cm*			<.0001
Yes	244 (31.0)	53 (0.5)	
No/Unknown	543 (69.0)	11074 (99.5)	
Disseminated symptoms*			<.0001
Yes	415 (52.7)	716 (6.4)	
No/Unknown	372 (47.3)	10411 (93.6)	

* Statistically significant with $P \leq 0.05$ between reportable vs. not reportable

**High incidence state = 10 or more cases per 100,000 in 3 consecutive years

**Low incidence state < 10 cases per 100,00 in 3 consecutive years

Factors associated with reportable cases:

The results for each predictive model were presented for a single training set. (Table 1.3). Moreover, results were similar across all training sets. Model 1 (*lab*) showed that the odds of being reportable was 18.26-fold (95% CI: 14.48 - 23.02) higher for a case-report with a positive IgG lab result, compared to a case-report that had a negative IgG lab result. No IgG WB performed (OR: 0.38, 95% CI: 0.31 – 0.47) was associated with a decreased odds of being a reportable case.

Model 2 (*lab, dem, tick*) showed that the odds of being reportable case was 17.91-fold (95% CI: 14.00 - 22.90) higher for a case-report with a positive IgG WB compared to a negative IgG WB. The odds of being a reportable case was higher within age category of 0 to 14 years (OR: 2.06, 95% CI: 1.51-2.81) compared to age category 15 to 29 years. The odds of being a reportable case was higher among males (OR: 1.96, 95% CI: 1.64 – 2.36) than females. The odds of being a reportable case was higher among being reported in the summer (OR: 3.60, 95% CI: 2.69 - 4.8), spring (OR: 1.74, 95% CI: 1.26– 2.40) and fall (OR: 1.57, 95% CI: 1.14 – 2.16) compared to winter. The odds of being a reportable case was higher with the addition of adult tick data (OR: 1.23, 95% CI: 1.08 - 1.41). All variables were statistically significant. The age category 76+ years (OR: 0.44, 95% CI: 0.25 – 0.78) and no IgG WB performed (OR: 0.36, 95% CI: 0.29 – 0.45) were associated with a decreased odds of being a reportable case.

Model 3 (*lab, dem, tick, clinical*) showed that the odds of being reportable case was 23.7-fold (95% CI: 17.1 - 32.8) higher for a case report with a positive IgG WB compared to a negative IgG WB. The odds of being a reportable case was higher among males (OR: 2.17, 95% CI: 1.72

– 2.73) than females. The odds of being a reportable case was higher among being reported in the summer (OR: 3.55, 95% CI: 2.47 - 5.11), spring (OR: 1.93, 95% CI: 1.29 – 2.88) and fall (OR: 1.92, 95% CI: 1.29 – 2.86) compared to winter. The odds of being a reportable case was higher among patients with a physician diagnosed EM >5cm (OR: 189.25, 95% CI: 128.11 - 279.56) or had had one or more disseminated symptoms (OR: 12.47, 95% CI: 9.70 - 16.04) compared to no EM or disseminated symptoms. All variables were statistically significant. Age and tick information did not inform the model as they were no longer statistically significant. No IgG WB performed (OR: 0.68, 95% CI: 0.51 – 0.92) was associated with a decreased odds of being a reportable case.

Model 4 (*lab, dem, tick, travel*) showed that the odds of being reportable case was 18.88-fold (95% CI: 13.59 – 26.24) higher for a case report with a positive IgG WB compared to a negative IgG WB. The odds of being a reportable case was higher among males (OR: 1.57, 95% CI: 1.26 – 1.97) than females. The odds of being a reportable case was higher among being reported in the summer (OR: 2.15, 95% CI: 1.53 – 3.02) and spring (OR: 1.51, 95% CI: 1.04 – 2.19) compared to being reported in the winter. The odds of being a reportable case was higher with travel to a high incidence state (OR: 3.10, 95% CI: 2.18 - 4.41) compared to travel to a low incidence state. The odds of being a reportable case was higher with the addition of adult tick data (OR: 1.68, 95% CI: 1.44 – 1.98). All variables were statistically significant. Age and did not inform the model as it was no longer statistically significant. No IgG WB performed (OR: 0.67, 95% CI: 0.51 – 0.88) was associated with a decreased odds of being a reportable case.

Table 1.3: Logistic regression analysis of the four predictive models based on a single training set

Variables	Model 1		Model 2		Model 3		Model 4	
	OR	**95% CI**	**OR**	**95% CI**	**OR**	**95% CI**	**OR**	**95% CI**
IgG Western Blot								
Negative IgG	Reference		Reference		Reference		Reference	
Positive IgG	**18.26**	14.48 - 23.02	**17.91**	14.00 - 22.90	**23.70**	17.14 - 32.77	**18.88**	13.59 - 26.24
Missing/No IgG	**0.38**	0.31 - 0.47	**0.36**	0.29 - 0.45	**0.68**	0.51 - 0.92	**0.67**	0.51 - 0.88
Age								
0 yrs - 14 yrs			**2.06**	1.51 - 2.81	1.48	0.99 - 2.20	1.40	0.96 - 2.04
15 yrs - 29 yrs			Reference		Reference		Reference	
30 yrs - 44 yrs			0.91	0.68 - 1.22	0.85	0.59 - 1.23	0.88	0.62 - 1.25
45 yrs - 59 yrs			0.96	0.73 - 1.27	0.91	0.64 - 1.29	0.96	0.68 - 1.34
60 yrs - 75 yrs			0.99	0.73 - 1.34	1.41	0.72 - 1.53	0.88	0.61 - 1.27
76 yrs or older			**0.44**	0.25 - 0.78	0.76	0.40 - 1.44	0.58	0.29 - 1.16
Sex								
Male			**1.96**	1.64 - 2.36	**2.17**	1.72 - 2.73	**1.57**	1.26 – 1.97
Female			Reference		Reference		Reference	
Season								
Fall			**1.57**	1.14 - 2.16	**1.92**	1.29 - 2.86	1.40	0.96 - 2.04
Winter			Reference		Reference		Reference	
Spring			**1.74**	1.26 - 2.40	**1.93**	1.29 - 2.88	**1.51**	1.04 - 2.19
Summer			**3.60**	2.69 - 4.81	**3.55**	2.47 - 5.11	**2.15**	1.53 - 3.02
Tick								
Adult MIP			**1.23**	1.08 - 1.41	1.14	0.96 - 1.34	**1.68**	1.44 – 1.98
Clinical								
Negative/Missing EM					Reference			
Positive EM					**189.25**	128.11 - 279.56		
Disseminated Symptoms								
Negative/Missing					Reference			
Positive					**12.47**	9.70- 16.04		
Travel								
Low Incidence State							Reference	
High Incidence State							**3.10**	2.18 - 4.41
No/Missing Travel							**0.03**	0.03 - 0.05

Bold OR estimates = statistically significant (95% CI does not include 1)

Performance characteristics of each model:

Performance characteristics, averaged across test sets, of each model are shown in Table 1.4. All models had good specificity and negative predictive values (NPV) greater than 95%. However, all models had low sensitivities with model 3 (*lab, dem, tick, clinical*) having the best sensitivity (62.56%) compared to all the other models. The accuracy for model 3 (*lab, dem, tick, clinical*) was 96.54% which was the highest compared to all the other models. Model 4 (*lab, dem, tick, travel*) performed comparably well to model 3 in terms of specificity, accuracy and the AUC (Table 1.4). Overall, models 3 (*lab, dem, tick, clinical*) and 4 (*lab, dem, tick, travel*) performed well in terms of their model characteristics.

Table 1.4: Average characteristic of each model

	Model 1 (Lab)	**Model 2** (Lab, Dem, Tick)	**Model 3** (Lab, Dem, Tick, Clin)	**Model 4** (Lab, Dem, Tick, Travel)
Sensitivity	50.17	37.26	62.56	49.76
Specificity	97.35	98.60	98.95	98.96
Positive Predictive Value (PPV)	57.27	65.58	80.80	77.39
Negative Predictive Value (NPV)	96.50	95.69	97.39	96.53
Accuracy	94.22	94.67	96.54	95.71
Area Under the Curve (AUC)	0.788	0.841	0.942	0.943

Average was calculated across the 10 test sets for each model

Table 1.4: Average characteristics of each model provides the test characteristics (sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), accuracy, and AUC.

Figures 2 and 3 are ROC curves of models 1-4 for each of the 10 test sets. The average AUC was calculated across all 10 test sets for each of the models. Models 1(*lab only*) and 2 (*lab, dem, tick*) represent the two models that require no follow up, as all information are directly reported to the CalREDIE system. The ROC analysis demonstrated that model 3 (*lab, dem, tick, clinical*) and model 4 (*lab, dem, tick, travel*) had the two highest AUC (Table 1.4) but they require further follow up to collect clinical and travel information (Table 1.4, Figures 1.1, 1.2)

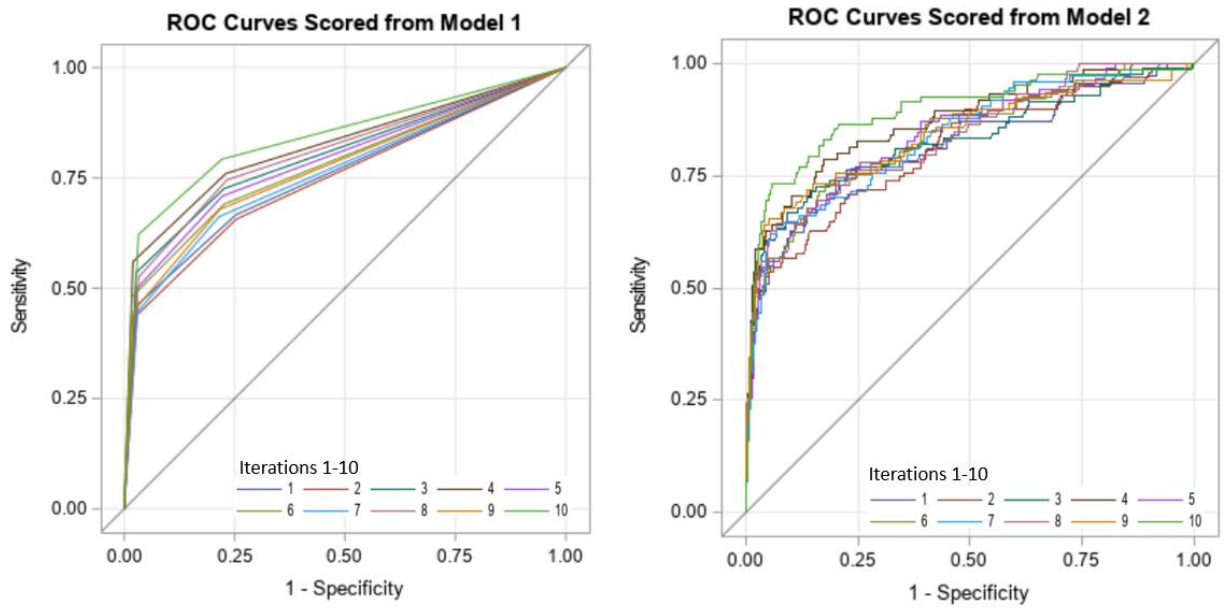

Figure 1.1: ROC curves for models 1 and 2: Represents the ROC curves of models 1 and 2 for each of the 10 test sets used in cross-validation. Models 1 and 2 do not require further investigation, all variables are automatically reported in CalREDIE

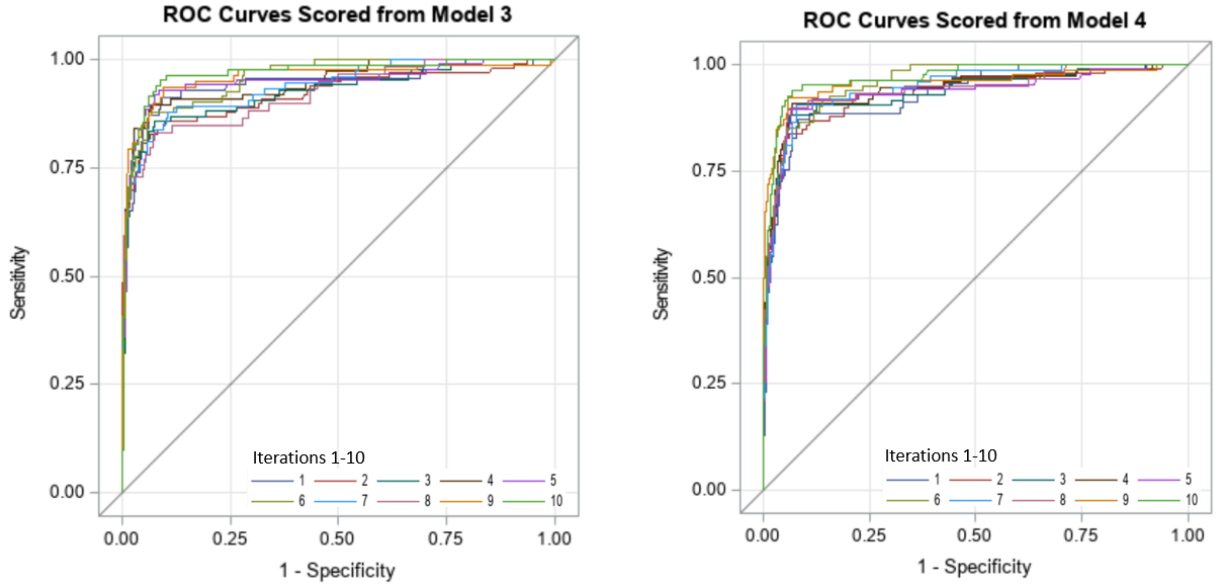

Figure 1.2: ROC curves for models 3 and 4: Represents the ROC curves of models 3 and 4 for each of the 10 test sets used in cross-validation. Models 3 and 4 require further investigation to obtain clinical and travel information

Discussion:

This study evaluated new approaches to minimize the information necessary to correctly classify a reported case of Lyme disease in California. The results of this study demonstrated that predictive modeling approaches were insufficient for estimating Lyme disease cases in California, a low incidence state for Lyme disease. Full investigations of Lyme disease case reports are crucial to accurately classify a case. Estimation sampling approaches was implemented in several high incidence states to reduce the burden of traditional surveillance case investigations for Lyme disease which proved successful (15, 16).

The results of this study showed that all models had very low sensitivities, which meant that each of the models missed 37% to 63% of case reports that are reportable. In general, a good diagnostic test, or in this case predictive model is characterized by a high true positive rate (sensitivity) and low false positive rate (1 - specificity) (28). The value of good sensitivity is approximately 80% (24). Each of the four predictive models had true positive values (sensitivity) less than 80% and false positive values (1 - specificity) less than 98%. These values indicated that the models were not able accurately predict reportable cases. Positive and negative predictive values are dependent upon the prevalence of disease in that population evaluated (25, 29). The positive predictive values for three of the four models were less than 80% except for model 3, which had the addition of clinical information. These values indicated a lower percentage of predicted cases that were actually reportable. Since the prevalence of Lyme disease in California is low at 0.2 confirmed cases per 100,000 a low positive predictive value for each model was expected. However, the four predictive models had an accuracy between 94% to 96%, which was largely driven by the high percentage of non-reportable cases, which all models

did well in predicting. An ROC curve was created for each of the 10 test sets for each model that assessed the accuracy and allowed for visual examination of each of the four predictive models (29). Models 3 and 4 performed equally well with an AUC at 0.942 and 0.943 respectively, however each of the models had poor sensitivities and poor positive predictive values.

Surveillance data in California from CalREDIE had missing information as many case reports are not fully investigated which vary across LHJ. Nevertheless, the surveillance data collected still provides valuable information on the epidemiology of Lyme disease in California. Surveillance data with its inherent limitations do provide important information in understanding the epidemiology of Lyme disease in the United States (4). We looked at six years of Lyme disease surveillance data from California, and the incidence was highest among the age group 45–59 yrs accounting for 22% of all reportable cases, with the age group 0 -14 yrs accounting for 19.6% of all reportable cases. We also saw that the age groups 30- 44 yrs and 60 -74 yrs accounted for 19.3% and 19.2% of reportable cases respectively, probably due to the way age was categorized. Over 50% of reportable cases were male; however, females accounted for over 60% of all case records being reported for Lyme disease. These trends are also seen in the United States, where there is a bimodal age distribution with peaks at 5 - 9 yrs and 50 - 55+ yrs with a male dominance (19, 30).

There are several limitations to this study, first surveillance data obtained from CalREDIE were used for each of the four predictive models and as the standard to which we compared our predicted models against as it is the most complete source of data estimating incidence and trends of Lyme disease in California. Another limitation, the variation in surveillance practices

between LHJ in California. Surveillance practices vary widely between counties in California; some counties do not investigate any Lyme disease case reports while others investigate all Lyme case reports. The variation in surveillance practices can result in the incidence of Lyme disease being underreported. A study done on estimating underreporting of Lyme disease in a low incidence state found that Lyme disease was underreported by at least 20% (31).

Another limitation is the use of tick surveillance data. This study used adult tick surveillance data only as a predictor because nymphal tick data were collected less consistently during the time period of 2000 – 2019 compared to adult tick data. However, the combination of adult and nymphal tick data may provide important information, since nymphal ticks in California pose the highest risk of Lyme disease transmission to humans in California (32-34). The density of host-seeking infected nymphs may be a better predictor of human diseases, but tick surveillance of *I. pacificus* overall is a poor indicator of human disease risk (35). This may relate to spatial heterogeneity in where ticks are found and where people spend time outdoors with a combination of human behaviors (35, 36). As the surveillance of nymphal ticks becomes more common, future models should assess the impact of these data on prediction of Lyme disease.

Our study results may not be generalizable to other low-incidence states for Lyme disease, since we used California's surveillance data as the training and testing sets for validation of our models. An outside data source, such as surveillance data from another low incidence state should be considered in the future to further evaluate the prediction models. Estimation sampling performed in high incidence states had several counties or states participating for external validation and generalizability to other high incidence states (15, 16).

Conclusion:

Each of the four predictive models had sensitivities lower 80% and was insufficient at accurately capturing reportable cases. The results showed that all four predictive models would underestimate the true incidence and therefore would not be a good approximation of the incidence of Lyme disease in California. We anticipate that the results from this research will start to address the burden of the increasing numbers of LD reports in a consistent way across jurisdictions. Various methodologies can inform state health department in low incidence states, such as the Californian Department of Public Health, on strategies to enhance surveillance practices for Lyme disease. Further research is needed to evaluate the balance of effort to collect necessary information to classify a case report consistently across all health jurisdictions with the return of pertinent epidemiologic information that meets the primary goals of surveillance.

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Chapter 2: Assessment of physician's knowledge and practice for Lyme disease in a low-incidence state

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Abstract:

Lyme disease (LD), caused by the bacterium *Borrelia burgdorferi,* is transmitted to humans *in California* through the bite of infected blacklegged ticks (*Ixodes pacificus*). The incidence of Lyme disease in California is low: approximately 0.2 cases per 100,000 population. However, California's unique ecological diversity contributes to focal, highly endemic regions which result in local areas with high human disease risk. The diagnosis of LD can be challenging in California because the prior probability of infection is generally low, symptoms are often non-specific, and interpretation of laboratory tests and related follow-up can be complicated. Therefore, California physicians need a high awareness of LD to recognize and diagnose the disease efficiently. This research aims to characterize physicians' knowledge and practices for testing and treatment of LD in California as a low-incidence state. We assessed physician's knowledge and practices related to the diagnosis of LD using an electronic survey distributed to physicians practicing in both higher and lower-incidence counties in California through mixed sampling methods. Our total sample size was 62: 26 (41.9%) from higher endemic counties and 36 (58.1%) from lower-incidence counties. We found that physicians in California would benefit from targeted education to improve test-ordering practices and test interpretation as well as increased awareness of California's unique ecology with high levels of focal endemicity, to improve recognition, diagnosis, and treatment of LD in California patients.

Introduction:

Lyme disease (LD), caused by the bacterium *Borrelia burgdorferi,* is transmitted to humans in California through the bite of infected western blacklegged ticks (*Ixodes pacificus*) (1-3). Lyme disease has been reportable in California since 1989 and became nationally notifiable in the United States in 1991 (4, 5). Lyme disease is the most common tick-borne disease in the US with over 30,000 cases reported annually. However, studies have shown that LD is subject to underreporting with estimates closer to 500,000 cases annually in the US by multiple data sources (4, 6, 7). The incidence of LD in California is low, with approximately 100 confirmed cases reported annually (0.2 cases per 100,000 population) (8). However, California's unique ecological diversity contributes to focal high-incidence regions, including the northwest coastal counties and northern counties along the foothills of the Sierra Nevada Mountain range, where human incidence ranges from 1.1 to 6.2 per 100,000 (9-12).

Signs and symptoms are divided into localized (3 to 30 days after exposure) and disseminated (days to months after exposure) (13-15). Early localized LD is characterized by an expanding erythematous rash ("erythema migrans" or EM) in about 70% to 80% of cases, typically larger than 5 cm, often with a well-demarcated outer border and central clearing ("Bull's eye") or diffuse with no blanching (4, 14). Early disseminated disease can include Lyme carditis, facial palsy, meningitis and, less commonly encephalitis (14). Late disseminated disease involves arthritis, described as severe joint pain and intermittent swelling typically of large joints, particularly the knees (4, 14, 16).

The clinical diagnosis of LD is based upon understanding of the clinical manifestations, laboratory results, and patient exposure histories (4, 17, 18). Recommended laboratory serologic testing by the Infectious Disease Society of America (IDSA) supports two-tiered testing (typically an enzyme immunoassay screen or immunofluorescent antibody test followed by a confirmatory western blot test, either IgM within first 30 days post onset or IgG thereafter) (19). If the EM is atypical or absent, and since subsequent symptoms of arthralgias and neuralgias are non-specific, testing strategies are not straight forward (14, 20). Effective diagnostic approaches depend on a health care provider's knowledge and awareness of LD (20).

The challenge for physicians diagnosing LD is determining whether testing is appropriate: when a patient [1] has symptoms that could yield a positive result if they are infected (i.e., should be some possibility of disseminated disease) and [2] enough time has elapsed since infection for an antibody response to develop (e.g., three to four weeks) (21, 22). These complexities, coupled with exposure in a low incidence state (California), can make the diagnosing of LD more complicated. This research will address an under-studied area of physician knowledge and practice of testing and treatment of LD in this low incidence state. Physician assessment in areas of the United States where the incidence of LD is high (19.7 - 106.6 per 100,000) suggests that physicians generally follow published guidelines (e.g. the IDSA guidelines for diagnosis and treatment of symptomatic LD (19) but are more likely to deviate from guidelines in the use of serologic testing and management of asymptomatic tick bites (20, 23, 24). It is not known if the aforementioned findings apply to low incidence areas. We surveyed physicians in California to determine if knowledge and practice of testing and treating LD differed among physicians practicing in higher endemic counties compared to lower endemic counties in an overall low

incidence state. The results of this study could help inform physicians and public health entities of subject areas on which to focus education to improve the testing and treatment of LD in a low incidence state.

Methods:

A survey of physicians practicing across 16 California counties was performed using a cross-sectional approach. The electronic questionnaire was developed to capture LD knowledge, testing, and treating practices of physicians. To increase survey responses, survey distribution was accomplished through multiple modalities. Initially, a medical marketing distribution company (MMS Distribution) was contracted to distribute the survey to physicians throughout California. The MMS healthcare provider database includes California-licensed physicians collected by the American Medical Association through the General Medical Education (GME) census. The physician survey by MMS Distribution was launched in March 2020 followed by three reminders, emailed in April, July, and August of the same year. Additional survey responses were sought through direct outreach to physicians at the University of California, Davis Medical Center (UCDMC), The University of California, San Francisco Medical Center (UCSF), Stanford Medical School, Palo Alto Medical Foundation (PAMF) and Dominican Hospital located in Santa Cruz, California. This study was approved by the Institutional Review Board (IRB) of the University of California Davis, protocol # 1388609-2.

Questionnaire:

The questionnaire was adopted from similar studies from high-incidence states such as Vermont and New Hampshire (25, 26), with modifications made to address the specific challenges faced

by physicians practicing in a low-incidence state like California. The modified questionnaire consisted of questions on physician practice characteristics (years of practice, medical specialty, practice setting, and county), questions addressing physician knowledge on LD such as understanding of the tick vector and its role in the medical diagnosis of LD, a question on serologic testing of LD, and patient scenario questions addressing laboratory (testing and interpretation of test results) and treatment of LD. The questionnaire was designed to take 5 to 10 minutes to complete. The survey was reviewed in consultation with three practicing California physicians. We piloted this survey by distributing to 16 California physicians to assess acceptability and ease of completion of the survey. A total of six (37.5%) of the pilot physicians responded. The final survey was adapted based on the responses of the pilot physicians and questions were added to assess practice of prophylaxis treatment for LD following a tick-bite in a low incidence state and inquire if physicians test patients to evaluate treatment success.

Sample area:

Sixteen California counties were chosen based on relative human LD incidence status. A "higher endemic" county in California was defined as a county with a 10-year incidence of > 1 case per 100,000 persons and a "lower endemic" county in California was defined as one with a 10-year LD incidence of < 1 case per 100,000 persons. The study was open to all physicians licensed to practice in California with specialties or subspecialties in internal medicine, family practice, pediatrics, infectious disease, rheumatology, and neurology. Physicians in some sub-specialties such as anesthesiology, obstetrics and gynecology, and radiology were not included as they were less likely to have a diagnostic encounter with a LD patient.

Sample size:

We planned to distribute 3,500 surveys to physicians in California. Power was calculated using an online calculator (https://stattools.crab.org/) for the comparison of the mean percentage of correct survey answers between physicians practicing in higher and lower endemic counties. The two-arm normal calculator was used, assuming a two-sided test, alpha=0.05, and equal numbers of physicians responding in higher endemic and lower endemic counties. Assuming a 10% response rate (n=350), we estimated 80% power to detect a difference in means as small as 0.3 standard deviations (SD) in the proportion of correct responses regarding knowledge of LD or testing protocol between physicians practicing in high and low endemic counties. Our study was designed before the global pandemic caused by SARS-CoV-2, but implementation occurred during the pandemic. A total of 3488 surveys were distributed, but a lower-than-expected survey response were obtained (23/3488 surveys) which motivated adoption of a mixed sampling scheme, where physician and physician groups were contacted opportunistically based on existing partnerships and health networks; through this added effort, an additional 39 survey responses were obtained.

Statistical analysis plan:

We obtained frequencies on all physician practice characteristic variables such as setting, specialty, and years of practice, as well as responses to LD practice questions and patient scenario questions. Bivariate analyses were performed to characterize practice attributes by county of practice located in either higher or lower endemic counties for LD. Ten questions were scored to test physician knowledge of testing and treatment for LD. Scoring involved calculating a percentage of the correct responses. The mean score (percentage correct) for each group of

respondents (defined by higher or lower endemic county) was compared by performing a Wilcoxon Rank Sum test, and an exact 95% confidence interval for the difference in means was computed. Individual scored questions were analyzed to assess areas of lower knowledge among either primary care or specialty care from high or low endemic counties for LD, a *P*-value ≤ 0.05 was considered statistically significant. All analyses were performed using SAS software version 9.4, SAS Institute Inc., Cary, NC, USA.

Results:

Sixty-four physicians responded to the survey; two physicians did not complete the survey and were not used in the final analysis. Our total sample size was 62: 26 (41.9%) from higher endemic counties and 36 (58.1%) from lower endemic counties. Using the .72 ratio of sample sizes between the two groups, and alpha equal to 0.05 for a two-sided test, we had 80% power to detect a difference between mean proportion correct in the high and low endemic counties with as small as 0.72 standard deviations.

Physician characteristics:

All participating physicians reported having Doctor of Medicine (MD) degrees. From higher endemic counties, 21 (80.8%) of the physicians practiced primary care including internal medicine, family practice, and pediatrics, and five (19.2%) practiced in specialty care including infectious disease, rheumatology, and neurology. In lower endemic counties, 13 (36.1%) physicians practiced primary care and 23 (63.9%) practiced in specialty care. In higher endemic counties, 11 (42.3%) physicians worked in the outpatient setting and eight (30.8%) worked in the hospital setting. In lower endemic counties, 18 (50.0%) physicians worked in a combination of

outpatient and hospital and 16 (44.4%) worked in an outpatient setting. Physicians from higher endemic counties had an average of 24.8 years of practice, ranging 2 to 50 years, compared with physicians from lower endemic counties with an average of 17.6 years ranging 1 to 45 years of practice. There were significant differences in physician characteristic in terms of specialties settings, and years of practice between higher and lower endemic counties ($p<0.05$) (Table 2.1).

Table 2.1: Practice characteristics of surveyed physicians

Physician Characteristic (N=62)	**High Endemic**	**Low Endemic**
	26 (41.9%)	36 (58.1%)
Specialty*		
Internal Med/Family Practice/Pediatrics	21 (80.8)	13 (36.1)
Infectious Disease/Rheumatology/Neurology	5 (19.2)	23 (63.9)
Setting*		
Outpatient	11 (42.3)	16 (44.4)
Hospital	8 (30.8)	2 (5.6)
Hospital/Outpatient	4 (15.4)	18 (50.0)
Other	3 (11.5)	0 (0.0)
Years of Practice*		
Mean (SD)	24.8 (12.9)	17.6 (11.9)
Range	2 years – 50 years	1 year – 45 years

* Statistically significant with $p<0.05$

Lyme disease practice:

Only six (23.1%) of the physicians practicing in higher endemic counties reported that LD was endemic in their county of practice while six (16.7%) physicians practicing in lower endemic counties reported that LD was endemic in their county of practice. The majority of physicians in both higher 20 (76.9%) and lower 29 (80.6%) endemic counties reported that they did not see an increase of LD patients in their practice. Sixteen (61.5%) physicians practicing in higher endemic counties and 26 (72.2%) physicians practicing in lower endemic counties reported that

patients have asked to be treated for LD even when their physician did not think their symptoms were caused by LD. Results further stratified by practice type were similar (Figure 2.1).

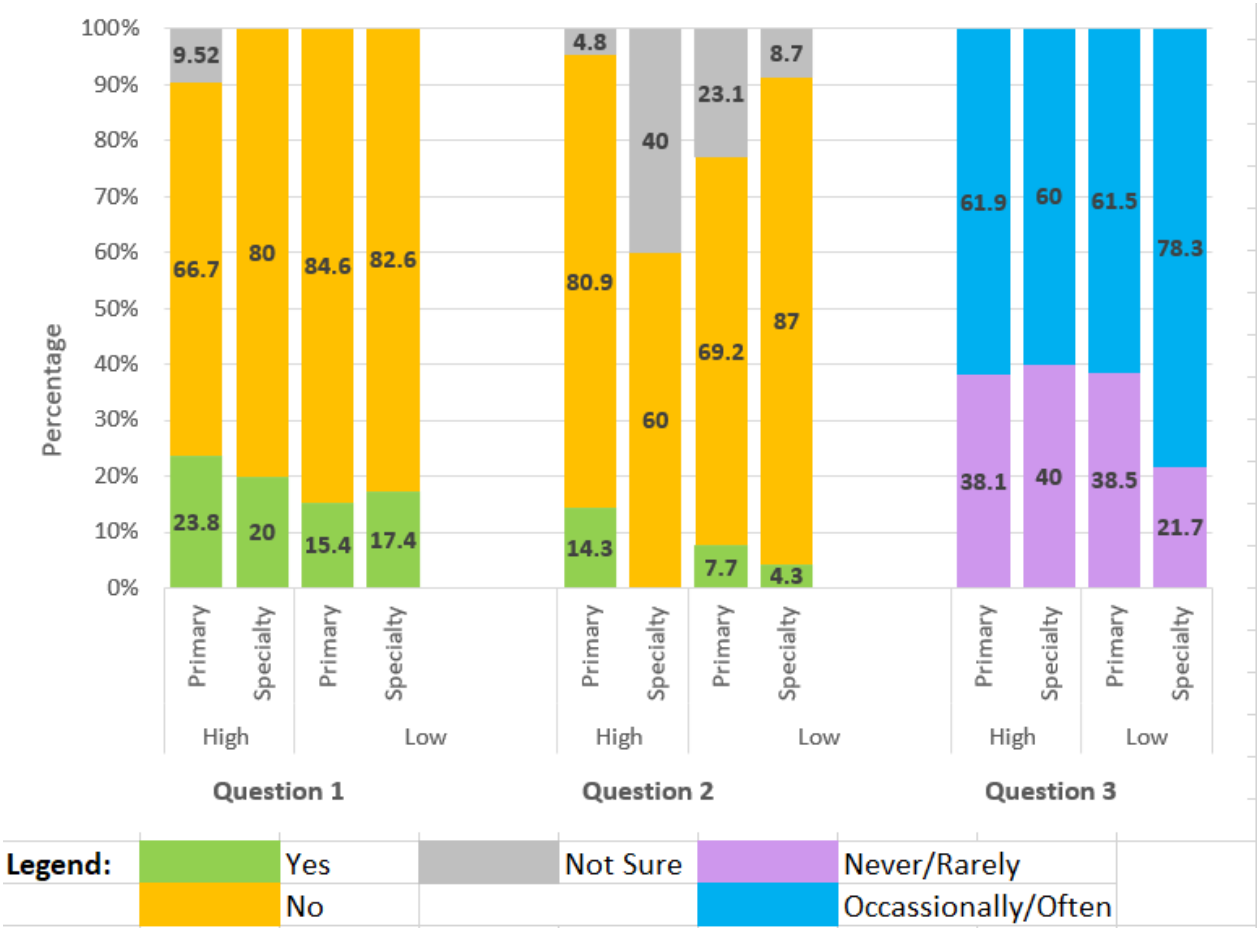

Figure 2.1: Lyme disease practice questions: Question 1: Within your geographic area of practice, would you consider Lyme disease (LD) endemic? Question 2: Have the number of LD cases increased among patients in your practice? Question 3: Have patients asked to be treated for LD though LD was the unlikely cause of their symptoms?

Physician survey (scored questions):

Only two (7.7%) physicians from higher endemic counties and two (5.6%) from lower endemic counties answered all scored practice questions correctly. Overall, the average percentage correct was 69.6% (standard deviation (SD) = 19.9, range = 20% - 100%) for higher endemic county

physicians and 73.6% (SD = 16.9, range = 30% - 100%) for lower endemic county physicians (Figure 2.2). Within primary care respondents in higher endemic counties, the percentage correct was 66.2% (SD =18.8, range = 20% - 100%), while within specialty care respondents, it was 84.0% (SD =19.5, range =50% - 100%). Similarly, within lower endemic counties, the percentage correct was 61.5% (SD =17.7, range = 30% - 80%) among primary care physician respondents and 80.4% (SD =12.2, range = 50% - 100%) from specialty care (Figure 2.3).

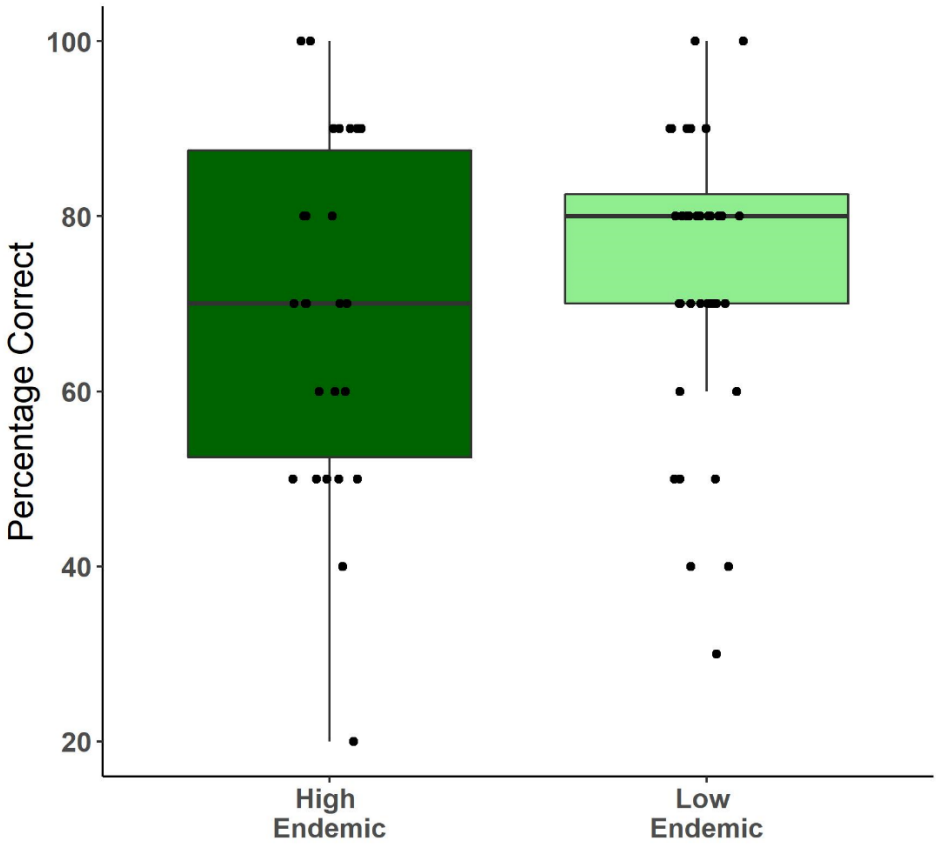

Figure 2.2: Percentage correct of scored questions by endemicity of counties. This figure shows the percentage correct of scored questions of physicians from higher and lower endemic counties for LD in California. There was no statistically significant difference among higher and lower endemic county physicians. *The top and bottom of the box represents the 75th and 25th percentiles respectively. The line through the box represents the median. The top whisker extends to the highest score that falls within the distance of 1.5 times the Interquartile range (IQR) = (Q3 – Q1) of the top of the box and the bottom whisker extends to the lowest score that*

falls within the distance of 1.5 times the IQR from the bottom of the box. Any points outside the whiskers are outliers.

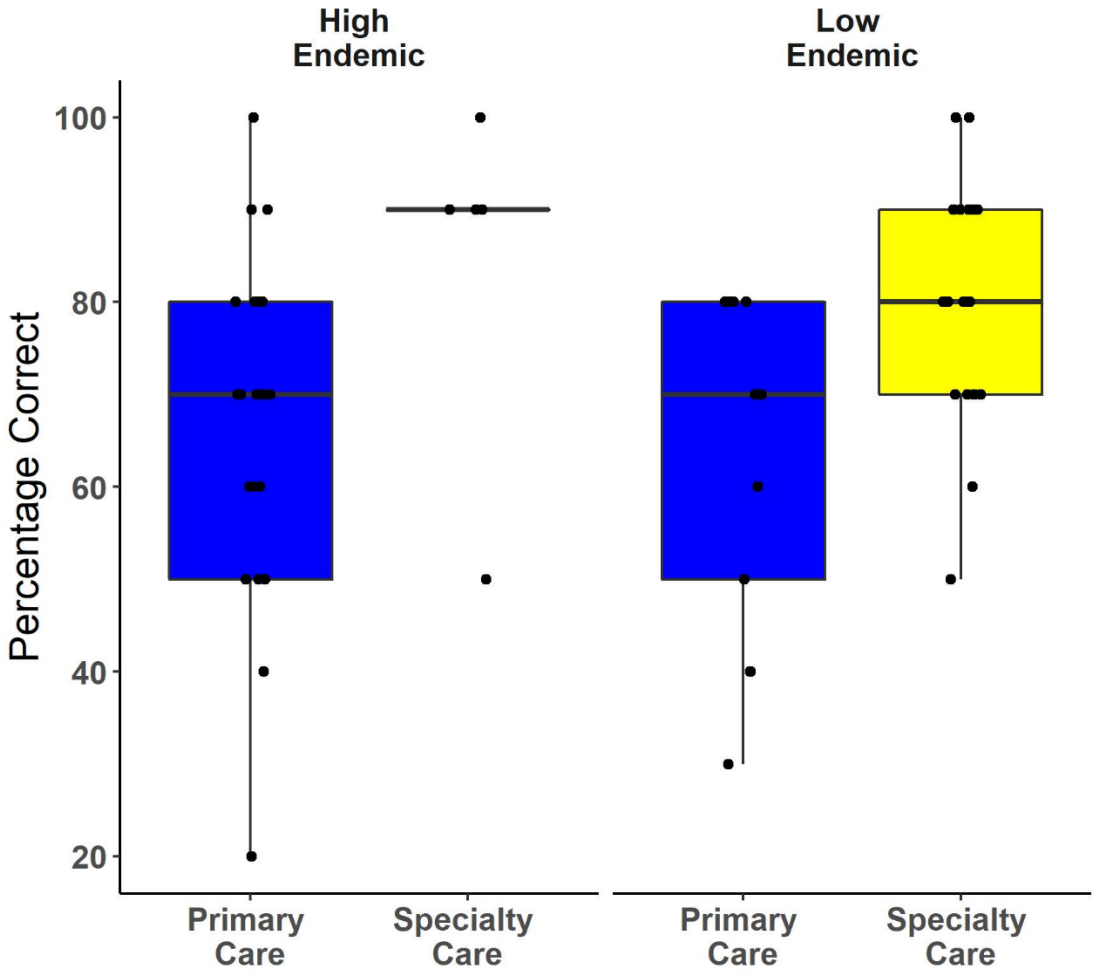

Figure 2.3: Percentage correct of scored questions by endemicity of counties and specialty. This figure represents the same set of scored questions as Figure 2.2 stratified by care type among higher and lower endemic counties for LD in California. There was a statistically significant difference between care type among the lower endemic physicians only.

Tick vector and diagnosis:

Over 73.0% (Exact 95% CI: 52.0 – 88.0) of higher endemic county physicians and 77.8% (Exact 95% CI: 61.0 – 90.0) of lower endemic county physicians responded that knowing the species of a tick recovered from a patient would be helpful in their medical diagnosis of LD, which was the

correct response. Sixty-nine percent (Exact 95% CI: 48.0 – 86.0) of higher endemic county physicians and 61.1% (Exact 95% CI: 43.0 – 77.0) of lower endemic county physicians correctly responded that if a tick recovered from a patient tested positive for *B. burgdorferi,* that positive tick result would be informative in their medical decision making about LD. However, both primary (71.4% Exact 95% CI: 48.0 – 89.0) and specialty (60.0% Exact 95% CI: 15.0 – 95.0) care physicians practicing from higher endemic counties as well as primary (84.6% Exact 95% CI: 55.0 – 98.0) and specialty (47.8% Exact 95% CI: 27.0 – 69.4) care physicians practicing from lower endemic counties incorrectly responded that if an asymptomatic patient had exposure to a positive tick for *B. burgdorferi*, it would inform their medical decision (Table 2.2).

Diagnostic testing:

There were two questions that focused on diagnostic testing for LD. Most physicians practicing in either primary care or specialty care from both higher and lower endemic counties correctly chose the laboratory diagnostic serologic test that satisfies the recommendation of the two-tier testing approach for the diagnosis of LD (Table 2.2). However, six physicians (23.1% Exact 95% CI: 9.0 – 43.5) practicing in higher endemic counties and five physicians (13.9% Exact 95% CI: 4.7 – 29.5) practicing in lower endemic counties incorrectly responded that they would perform PCR testing on the synovial fluid from the patient's swollen knee instead of two-tier testing on blood or sera (Table 2.2).

Interpretation of Lyme disease test results:

Three questions focused on interpretation of test results in three different scenarios. Over 90% of physicians practicing in either primary care or specialty care from both higher and lower

endemic counties responded correctly to the first scenario assessing interpretation of diagnostic test results in the presence of an EM (Table 2.2). The second scenario question assessed interpretation of diagnostic test results from a patient with lingering symptoms for over two years. Physicians practicing in specialty care tended to correctly answer this scenario whether they were practicing in high endemic 80% (Exact 95% CI: 28.4 – 99.5) or low endemic 95.6% (Exact 95% CI: 78.0 – 99.9) counties, while primary care physicians answered the question correctly less frequently with 66.7% (Exact 95% CI: 43.0 – 85.4) in higher endemic counties and 53.8% (Exact 95% CI: 25.1 – 80.8) in lower endemic counties answering correctly (Table 2.2). The percentage responding correctly differed between the specialty care and primary care settings in the lower endemic counties (p=0.005) (Table 2.2). The last scenario question assessed interpretation of the two-tier testing approach of the diagnosis of LD. Specialty care physicians from both higher 80.0%; (Exact 95% CI: 28.4 – 99.5) and lower endemic 82.6%; (Exact 95% CI: 61.2 – 95.1) correctly answered the interpretation scenario questions more than primary care physicians regardless of endemicity (higher endemic 42.9%; (Exact 95% CI: 21.8 – 66.0) and lower endemic 46.1%; (Exact 95% CI: 19.2 – 74.9). However, the difference was significant only in the lower endemic counties (p=0.035) (Table 2.2).

Treatment of Lyme disease:

There were three questions that focused on the treatment of LD. The first scenario question inquired if additional testing would be warranted to determine further treatment for LD in a patient that was already diagnosed and treated. All specialty care physicians in both higher and lower endemic counties correctly answered that question in the negative. However, only eight (61.5%; Exact 95% CI: 31.6 – 86.1) primary care physicians from lower endemic counties

responded correctly, a significantly lower percentage (p=0.003) than the specialty care physicians in those counties (Table 2.2). Most physicians practicing in primary and specialty care from both higher and lower endemic counties correctly responded that physicians should not treat a tick bite prophylactically (question 2) and that physicians should not treat long term arthritis with negative test for LD (question 3) (Table 2.2).

Table 2.2: Lyme disease patient scenario questions

Patient Scenario Questions		High Endemic		Low Endemic	
	Responses	**Primary**	**Specialty**	**Primary**	**Specialty**
Interpretation of Test Results		n (%)	n (%)	n (%)	n (%)
(1) A healthy patient with a history of daily hiking in the month of April and in an area where ticks are found, presents in your office with a rash resembling an erythema migrans that began 3 days earlier. You order a serologic test for Lyme disease which yields a negative result. Would you consider this negative test result definitive to rule out Lyme disease as the cause of this patient's rash?	Yes **No** Maybe	0 (0.0) **20 (95.2)** 1 (4.7)	0 (0.0) **5 (100.0)** 0 (0.0)	2 (15.4) **11 (84.6)** 0 (0.0)	1 (4.3) **21 (91.3)** 1 (4.3)
(2) A 45-year-old patient from Southern California presents with fatigue and difficulty concentrating for the past two years. The patient does not remember a tick bite or rash but occasionally gardens in the backyard. The patient has not travelled out of Southern California for the past two years. A Lyme disease test was ordered at the time of the visit and the results were: Equivocal EIA, positive IgM Western blot (2/3 bands), negative IgG Western blot (1/10 bands). What is your interpretation of these results?	**Unlikely to have LD** Likely to have LD Other	**14 (66.7)** 5 (23.8) 2 (9.5)	**4 (80.0)** 1 (20.0) 0 (0.0)	**7 (53.8) *** 5 (38.5) 1 (7.7)	**22 (95.6) *** 1 (4.3) 0 (0.0)
(3) How would you interpret this test result from a patient you tested for Lyme disease: Negative EIA, positive IgM western blot, and negative IgG western blot?	**Unlikely to have LD** Likely to have LD Other	**9 (42.9)** 9 (42.9) 3 (14.3)	**4 (80.0)** 1 (20.0) 0 (0.0)	**6 (46.1) *** 4 (30.8) 3 (23.1)	**19 (82.6) *** 1 (4.3) 3 (13.0)
Treatment of LD					
(1) A 35-year-old patient was diagnosed (based upon positive serology and compatible clinical symptoms) and treated for Lyme disease. Are additional serologic test for Lyme disease warranted after treatment?	Yes **No** Not Sure	1 (4.8) **17 (80.9)** 3 (14.3)	0 (0.0) **5 (100.0)** 0 (0.0)	1 (7.7) **8 (61.5) *** 4 (26.7)	0 (0.0) **23 (100.0) *** 0 (0.0)
(2) A patient presents to your clinic concerned with a tick bite received about 30 days ago. The patient has not travelled outside of CA, the patient has no symptoms, no laboratory testing performed to date, and normal examination findings. Which of the following describes your next action?	Treat for LD Treat tick bite prophylactically. **Do not treat LD** Other	0 (0.0) 0 (0.0) **20 (95.2)** 1 (4.7)	0 (0.0) 0 (0.0) **5 (100.0)** 0 (0.0)	0 (0.0) 1 (6.7) **12 (92.3)** 0 (0.0)	0 (0.0) 1 (4.3) **22 (95.7)** 0 (0.0)
(3) A patient presents with recurrent, asymmetric arthritis that began 3 months prior, involving large, weight-bearing joints. The patient has no history of an erythema migrans rash and has had multiple negative Western (IgM/IgG) blot test results for Lyme disease over the past 3 months. The patient does not recall a tick bite, but the patient spends a lot of time outdoors. Which of the following describes your next action?	Treat for LD **Do not treat LD** Other	0 (0.0) **19 (90.5)** 2 (9.5)	0 (0.0) **5 (100.0)** 0 (0.0)	1 (7.7) **12 (92.3)** 0 (0.0)	1 (4.3) **21 (91.3)** 1 (4.3)
Vector and diagnosis					
(1) If you submitted a tick recovered from a patient for identification, would knowing the tick species inform your medical decision-making about Lyme disease?	**Yes** No Not Sure	**14 (66.7)** 5 (23.8) 2 (9.5)	**5 (100.0)** 0 (0.0) 0 (0.0)	**10 (76.9)** 1 (7.7) 2 (15.4)	**18 (78.3)** 4 (17.4) 1 (4.3)
(2) If you submitted a tick recovered from a patient to be tested for *Borrelia burgdorferi*, would the tick testing result inform your medical decision-making about Lyme disease?	Yes **No** Not Sure	15 (71.4) **5 (23.8)** 1 (4.8)	3 (60.0) **1 (20.0)** 1 (20.0)	11 (84.6) **0 (0.0)** 2 (15.4)	11 (47.8) **5 (21.7)** 7 (30.4)
Diagnostic Testing					
(1) What Lyme disease diagnostic tests do you commonly order for a suspected Lyme disease patient? **	**Western Blot IgG** **EIA/IFA/ELISA** PCR (Blood Tissue) Culture CD57 **Western Blot IgM** PCR (Synovial Fluid) Plasmid Other	**11 (52.4)** **17 (80.9)** 4 (19.0) 0 (0.0) 0 (0.0) **10 (47.6)** 0 (0.0) 0 (0.0) 3 (14.3)	**5 (100.0)** **4 (80.0)** 0 0.0) 0 (0.0) 1 (20.0) **4 (80.0)** 0 (0.0) 0 (0.0) 1 (20.0)	**8 (61.5)** **6 (46.1) *** 2 (15.4) 0 (0.0) 0 (0.0) **7 (53.8)** 0 (0.0) 1 (7.7) 3 (23.1)	**18 (78.3)** **20 (87.0) *** 3 (13.0) 0 (0.0) 0 (0.0) **15 (65.2)** 0 (0.0) 0 (0.0) 3 (13.0)
(2) A 50-year-old patient from Northwest California presents with a swollen, erythematous knee for the past week. The patient does not remember a tick bite or rash but is active outdoors and went on a hiking trip to the coastal foothills two months ago. You suspect Lyme disease. Which of the following testing approaches would yield the most diagnostic information?	No Testing Needed Order EIA only Order WB only **Two Tier Testing** PCR joint fluid Other	3 (14.3) 0 (0.0) 0 (0.0) **11 (52.4) *** 5 (23.8) 2 (9.5)	0 (0.0) 0 (0.0) 0 (0.0) **4 (80.0) *** 1 (20.0) 0 (0.0)	0 (0.0) 0 (0.0) 2 (15.4) **9 (69.2)** 1 (7.7) 1 (7.7)	1 (4.3) 0 (0.0) 0 (0.0) **17 (73.9)** 4 (17.4) 1 (4.3)

LD = Lyme disease
Bold = Correct response
* Statistically significant at alpha = 0.05, comparing primary care to specialty care

Discussion:

This study's purpose was to characterize physician knowledge and practice about LD in a low incidence state in the US, using similar high incidence state studies as a framework. Though California is a low incidence state, we felt it useful to classify our physician respondents based on Lyme endemicity of their county of practice because we hypothesized that those physicians practicing in higher endemic California counties would perform comparably to physicians practicing in high incidence states. While our low response rate precluded us from detecting significant differences between these two county classifications, by focusing on care type in each area, we were able to identify some interesting patterns in knowledge of testing and treating patients in the context of LD. Our survey results demonstrated that physicians in California could benefit from targeted education to improve test-ordering practices and test interpretation as well as increased awareness of California's unique ecology with high levels of focal endemicity.

Most physicians from both higher and lower endemic counties did not think LD was endemic in their county of practice, even among those physicians who practiced in areas of higher endemicity. Even though California has a low incidence of LD, most physicians would benefit from an increased awareness that California has focal areas of higher endemicity which may pose a risk of human infection for residents living or recreating in these areas (11, 12, 27). Insufficient knowledge on where infected ticks is found in California can be problematic in the physician's overall care of their patients, affecting testing, diagnosis, and treatment of LD.

The diagnosis of LD in patients with compatible clinical symptoms and recent travel to high incidence states is relatively straight forward (28). However, patients with a locally acquired tick

bite from a low incidence area may pose a diagnostic challenge further complicated by a decreased positive predictive value in the serologic testing in a low pre-test probability setting (29). We found that our physicians in this study deviated from IDSA national guidelines in diagnostic testing for LD when patients sought care for both symptomatic disease and asymptomatic tick bites, whereas physicians from high incidence states were more likely to deviate from diagnostic testing guidelines in patients with asymptomatic tick bites only (24). Serologic testing for LD in a low incidence state performs well under appropriate settings, such as obtaining complete medical, travel and exposure history of the patient. The importance of obtaining a travel history should be emphasized, as it allows physicians in low-incidence states to recognize patients at lower or higher risk of disease (28). Over-testing and overdiagnosis are common in the United States and California (30, 31), potentially exacerbated by patient demand to be treated though LD unlikely to be the cause of complaint. Physicians should order serological testing for LD judiciously and in accordance with IDSA national guidelines (30).

Although PCR testing for the detection of spirochetal DNA on appropriate clinical samples (such as synovial fluid) has an overall better sensitivity then serologic testing (32), it should only be performed when analytical and clinical validity has been demonstrated (19). The PCR test is not a reliable standalone blood test as there are many limitations to this testing method (19, 33, 34). To date, there are no FDA approved PCR tests for the diagnosis for LD (19). However, it is interesting to note that a small proportion of physicians practicing in higher and lower endemic counties for LD chose PCR testing of blood as the preferred diagnostic test.

The interpretation of serologic antibody testing for LD could be a critical educational opportunity for our physicians in California. A little more than half of primary care physicians practicing in both higher and lower endemic counties struggled with discerning a false-positive IgM in a patient with longstanding symptoms. These results are similar to what was found in high incidence states (25). The diagnostic serologic tests available for confirmation of human LD has variable sensitivity and specificity and is dependent on the stage of infection (35, 36). Use of IgM testing is relevant for detecting early disease, during the first 30 days of infection, after which IgG tests should be used (35, 36). Also, about 50% of our primary care physicians practicing in both higher and lower endemic counties had misinterpreted the results of positive IgM western blot in the presence of negative EIA/IFA and negative IgG western blot. Several studies have shown that a positive IgM western blot in the presence of a negative screening test and IgG western blot accounted for more than 50% of all false positives results (30, 37). The IDSA national guidelines recommends a two-tiered approach to LD serologic testing to achieve the highest sensitivity and specificity (19, 38, 39). The first tier consists of an immunoassay using whole-cell, recombinant, or synthetic peptide antigens or, rarely, an immunofluorescence assay (IFA) (40). If results of the first test are positive or indeterminate, supplementary western blots are recommended to increase testing specificity (39). The IgM western blot is a valuable second tier test for early LD, given the relatively slow appearance of IgG antibodies (30, 37, 41, 42). However, the positive predictive value of the IgM western blot is low in patients who lack clinical features of Lyme disease (41). Overall, specialty physicians performed better on interpretation of test results for LD, as most of the specialty physicians in our study were infectious disease doctors.

A single 200-mg dose of doxycycline has been shown to reduce the risk of LD after the removal of an attached *Ixodes scapularis* tick in high incidence states where the local infection rate is >20% (43). The majority of our physicians practicing from both higher and lower counties correctly responded that they would not treat their patients prophylactically to prevent LD following presentation with a tick bite in a low incidence state. However, survey results from the CDC found that 48% of physicians practicing in a low incidence state would prescribe tick bite prophylaxis (43). Antibiotic prophylaxis in low incidence states is not recommended due to low risk of infection after a single tick bite versus the risk of associated side effects of the antibiotic. (43)

There are limitations to this study. This research was performed during a global pandemic (SARS-CoV-2) so the number of physicians responding was extremely low. Physicians are a challenging group to survey for various reasons, ranging from busy schedules to frequent survey requests (44) however, trying to gather physician participation amidst a global pandemic becomes especially problematic. It is also unknown whether those physicians who responded to the survey systematically differed from those physicians who did not respond to the survey. The small sample size did not detect a difference between physicians practicing in higher and lower endemic counties which led to uncertainty of our estimates. However, despite the small sample size, we received a similar proportion of physicians practicing from higher and lower endemicity counties.

Conclusion:

To the extent that our surveyed physicians represent California physicians, a greater awareness of California's variable ecology and its impact on LD infection risk to humans would be beneficial to California physicians. The risk of LD varies in California and this variation in risk can impact diagnostic testing and interpretation. Physicians in California could benefit from further targeted education to better understand disease risk in California and to improve recognition of symptoms and appropriate use and interpretation of serologic testing. Future physician knowledge and practice of LD studies of a larger scale would be helpful in understanding LD medical approaches among other low incidence states.

Implications of policy and practice:

1) In a low incidence state like California, insufficient knowledge on geographic risk for Lyme disease may impact a physician's overall care of their patients, affecting testing, diagnosis, and treatment of LD.

2) Medical education efforts on Lyme disease in California should focus on topics such as where human risk is greatest and diagnostic testing protocols for a low incidence state.

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Chapter 3: *Borrelia burgdorferi* and *Borrelia miyamotoi* Seroprevalence in California Blood Donors

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Short Title: *Borrelia* spp. In California Blood Donors

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Abstract:

The western blacklegged tick, *Ixodes pacificus*, an important vector in the western United States of two zoonotic spirochetes: *Borrelia burgdorferi* (also called *Borreliella burgdorferi*), causing Lyme disease, and *Borrelia miyamotoi*, causing a relapsing fever-type illness. Human cases of Lyme disease are well-documented in California, with increased risk in the north coastal areas and western slopes of the Sierra Nevada range. Despite the established presence of *B. miyamotoi* in the human-biting *I. pacificus* tick in California, clinical cases with this spirochete have not been well studied. To assess exposure to *B. burgdorferi* and *B. miyamotoi* in California, and to address the hypothesis that *B. miyamotoi* exposure in humans is similar in geographic range to *B. burgdorferi*, 1,700 blood donor sera from California were tested for antibodies to both pathogens. Sampling was from high endemic and low endemic counties for Lyme disease in California. All sera were screened using the C6 ELISA. All C6 positive and equivocal samples and nine randomly chosen C6 negative samples were further analyzed for *B. burgdorferi* antibody using IgG western blot and a modified two ELISA test system and for *B. miyamotoi* antibody using the GlpQ ELISA and *B. miyamotoi* whole cell sonicate western blot. Of the 1,700 samples tested in series, eight tested positive for antibodies to *B. burgdorferi* (0.47%, Exact 95% CI: 0.20, 0.93) and two tested positive for antibodies to *B. miyamotoi* (0.12%, Exact 95% CI: 0.01, 0.42). There was no statistically significant difference in seroprevalence for either pathogen between high and low Lyme disease endemic counties. Our results confirm a low frequency of Lyme disease and an even lower frequency of *B. miyamotoi* exposure among adult blood donors in California; however, our findings reinforce public health messaging that there is risk of infection by these emerging diseases in the state.

Introduction:

California is considered a low incidence state for Lyme disease, defined as a state with a disease incidence of <10 confirmed cases/100,000 annually (1). The varied ecology results in some counties having higher endemicity for Lyme disease than others (2-4). The western blacklegged tick (*Ixodes pacificus*) is a common human-biting tick in California and is the principal vector for *Borrelia burgdorferi* sensu stricto, also called *Borreliella burgdorferi* and hereinafter referred to as *B. burgdorferi* (5). *B. burgdorferi* is the causative agent of Lyme disease in humans and animals in North America. Other potentially zoonotic spirochetes have been documented in the western blacklegged tick (6-8), most notably *Borrelia miyamotoi*, an emerging tick-borne pathogen that is in same genus as the agents of relapsing fever. It causes a febrile illness that occasionally may relapse (9, 10).

In California, the prevalence of *B. burgdorferi* is typically higher in nymphal *I. pacificus* ticks (~3-5%) than adult ticks (~1% or less) (11). The prevalence for *B. miyamotoi* is about the same (~1%) in both of these tick stages (12-16). *B. miyamotoi* is transmitted both transstadially and transovarially, which means that while the risk of exposure to *B. burgdorferi* is greater than *B. miyamotoi* after exposure to nymphal ticks, *B. miyamotoi* infection may occur after larval, nymphal, or adult tick exposure and thus extends the season of risk (11). The distribution of *B. miyamotoi* in *I. pacificus* ticks appears to be similar to that of *B. burgdorferi* and is most prevalent in coastal and foothill regions of northern California (11, 17). Despite ample evidence of *B. miyamotoi* in California ticks, including ticks that were recovered from humans (18), epidemiological information and case descriptions of *B. miyamotoi* infections in humans are lacking in California.

There are a handful of tick-borne relapsing fever cases caused by *Borrelia hermsii* reported each year in California (15). Although this spirochete is phylogenetically related to *B. miyamotoi* and shares common antigens (19), its ecology is quite distinct. It is vectored by the soft tick, *Ornithodoros hermsi*, which can be found in rodent nests built in cabins and houses in the mountains and foothill regions of the western United States (20). The possibility of human infections with *B. miyamotoi* in California comes from a recent study that identified seroreactivity to *B. miyamotoi* in an area highly endemic for Lyme disease in northern California. Seroprevalence was 12% – 14% in the study participants who were at high risk for tick-borne disease, although the GlpQ-based serologic assay used to test for *B. miyamotoi* antibody could not differentiate *B. miyamotoi* infection from *B. hermsii* (21). The authors surmised that ecology and known behaviors of study participants suggested that they were most likely exposed to *B. miyamotoi* rather than *B. hermsii* (21).

Even with a low prevalence of *B. burgdorferi* and *B. miyamotoi*, California residents are still at risk for tick borne relapsing fever. The purpose of the present study was to determine whether *B. miyamotoi* has a broader geographical range in California than previously demonstrated and to compare the seroprevalence of *B. burgdorferi* and *B. miyamotoi* over this larger range. We therefore assessed human exposure to *B. burgdorferi* and *B. miyamotoi* by testing 1,700 blood bank serum samples from both high and low Lyme disease endemic areas in California. As a broad California-based serosurvey, findings from this study should inform public-health messaging as well as future *B. burgdorferi* and *B. miyamotoi* research.

Material and Methods:

Study population:

We obtained 1,700 de-identified human sera samples from Creative Testing Solutions (www.mycts.org) consisting of human sera samples from blood banks. These included 941 samples from high endemic Lyme disease counties in California, defined as $\geq$ 1 case per 100,000 annually and 759 samples from low endemic Lyme disease counties, defined as < 1 case per 100,000 annually (Figure 3.1). Sample size calculations were performed using Ausvet Epi tools Epidemiological calculators, with sample size calculated based on the following assumptions: (i) an estimated human prevalence of 2% for Lyme disease in high endemic California counties and an estimated human prevalence of 1% for low endemic Lyme disease counties (11, 16); (ii) a C6 ELISA screening test with a sensitivity of 97% and a specificity ranging from 93% to 99% for antibodies to *B. burgdorferi*; and (iii) a desired precision of 0.012 with an α= .05 and 80% power. Inclusion criteria for blood donor serum samples included the zip code of the blood donor, sera that was non-reactive on screening assay for other infectious diseases including Hepatitis B or C, HIV 1 or 2, HTLV I/II, West Nile virus, Zika virus, and syphilis; and a minimum volume of at least 1ml. Samples were collected from April 2017 through June 2017, corresponding with typical seasonality for Lyme disease cases reported in California (11, 22). Information associated with the samples included general demographic information such as sex, age, ethnicity, county and zip code of the donor.

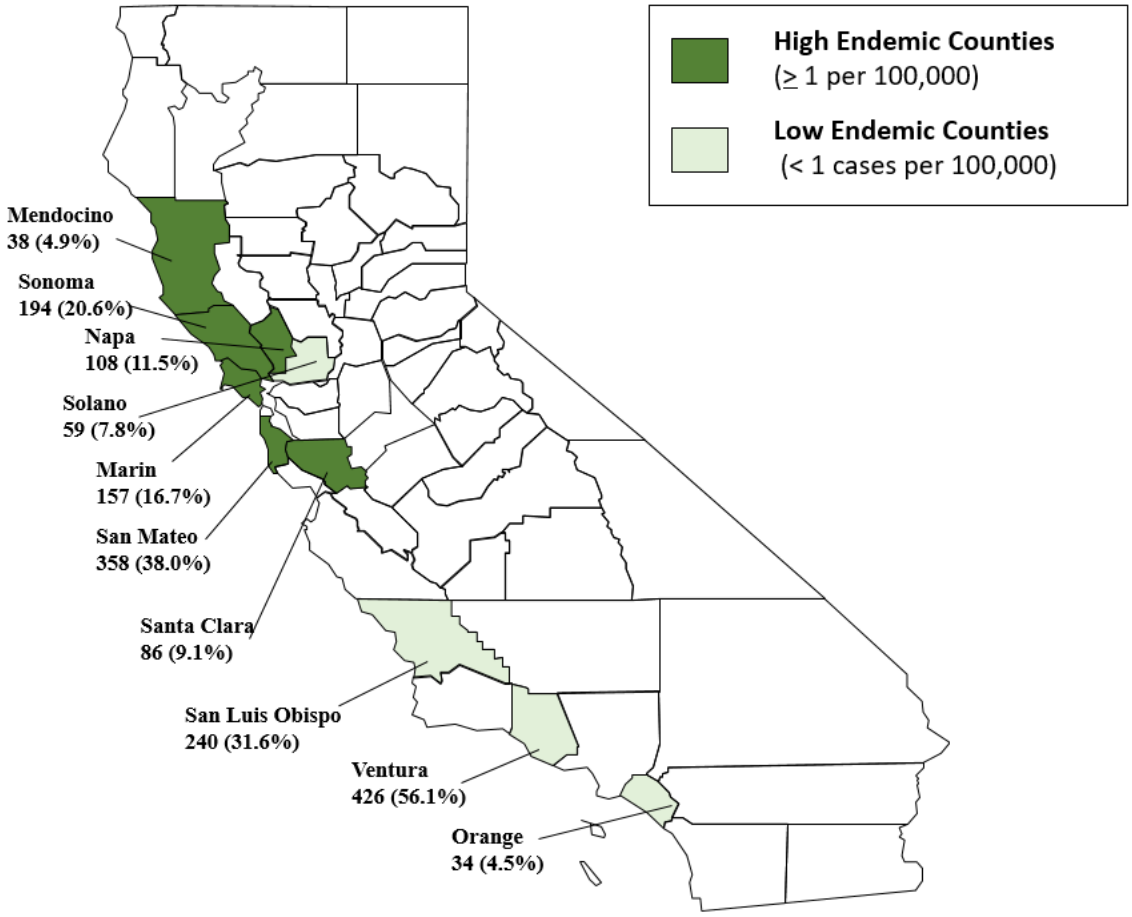

Figure 3.1: **Counties in California in which samples were collected**: The counties in dark green represent high endemic counties in California for Lyme disease and the counties in light green represent low endemic counties in California for Lyme disease. Number and percentages of sera samples by county are provided. Lyme disease incidence is based upon both historical human and tick data in California.

Ethics approval:

De-identified serum samples with associated demographic variables for each serum sample were submitted for this study. The study was determined to have exempt status by the Institutional Review Board, University of California Davis (Protocol Number 1090480-1, FWA No: 00004557).

Sample processing and storage:

Creative Testing Solutions shipped frozen one-ml aliquot sera samples on dry ice to the testing facility at the University of California, Davis (UCD). UCD stored all sera samples at -80°C until thawed in the refrigerator for one to five days until testing. By using the commercial C6 ELISA test as a screening tool, the full set of samples could be initially evaluated for reactivity to *B. burgdorferi* and *B. miyamotoi* since both of these agents react to this antigen (23, 24). The IgG western blot for *B. burgdorferi* and total Ig GlpQ ELISA and IgG western blot for *B. miyamotoi* were subsequently used to test all C6 positive/equivocal samples, as well as nine randomly selected C6 negative samples, to serve as controls (23, 25-27). Additionally, a recent FDA approved test system for detection of *B. burgdorferi* antibodies, referred to as the Modified Two-Tier Test (MTTT) (Zeus Scientific, New Jersey), was performed in accordance with the manufacturer's guidelines on the C6 positive/equivocal and C6 negative samples (28). After C6 screening (described below), all C6-positive/equivocal and the randomly chosen negative samples were separated into 250µl aliquots in coded tubes, re-coded to mask county of location, and shipped frozen to the Centers for Disease Control and Prevention (CDC). CDC stored all sera samples in a -80°C freezer until testing (Figure 3.2).

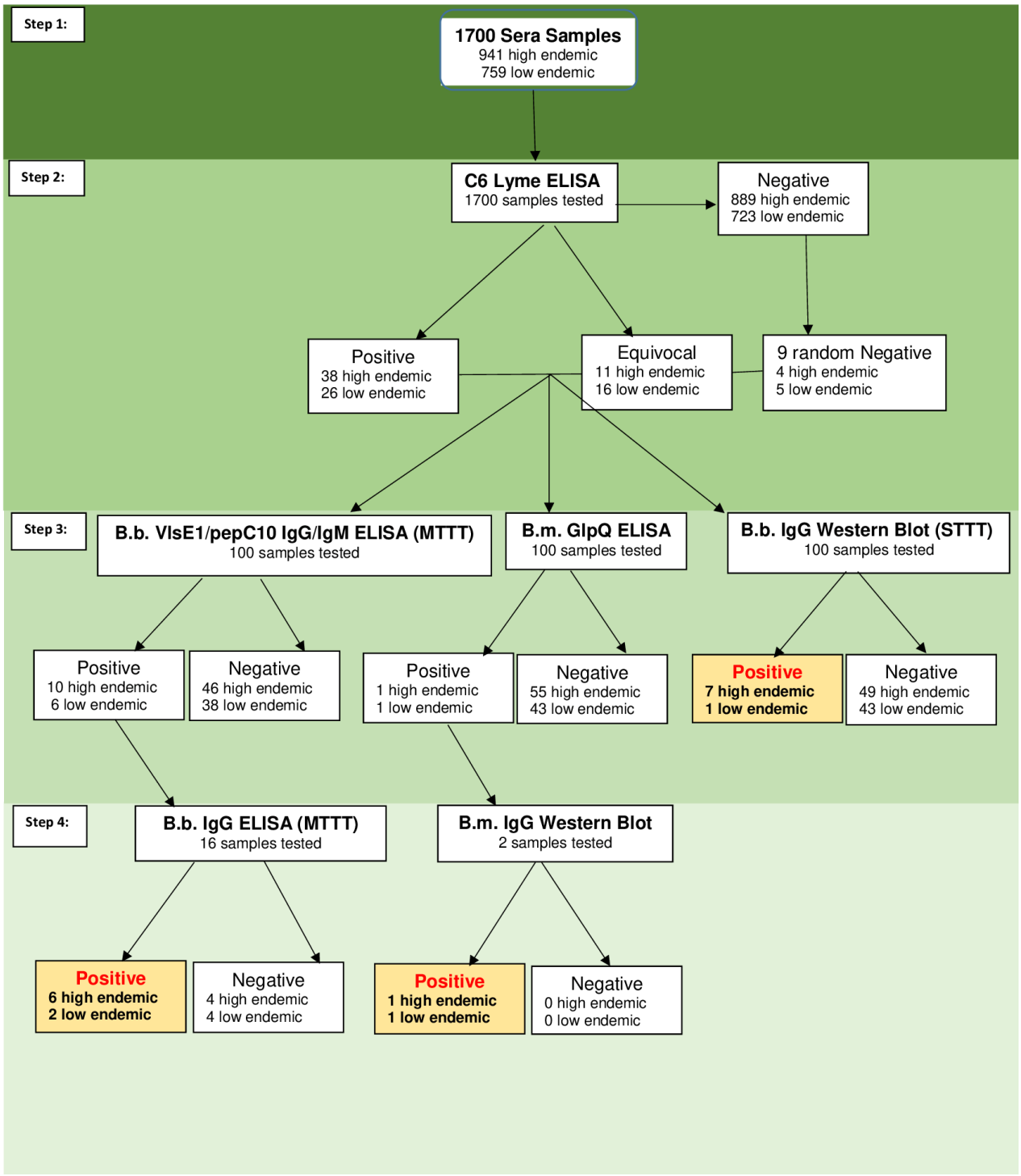

Figure 3.2: Flowchart of testing strategies: This figure depicts the testing strategy that was performed on the 1,700 blood donors from California. Each layer of the testing strategy explains the test performed and the number of samples tested.

Standard two-tiered testing:

All sera samples were screened using a C6 Lyme ELISA kit (Immunetics, Boston Massachusetts) performed in accordance with the manufacturer's recommendation. Testing was performed in duplicate for all specimens. A Lyme index value for each sample was calculated by dividing the average of the sample's OD values by the calibrator cutoff value. Positive and negative controls were provided by the manufacturer. Samples positive or equivocal by C6 Lyme ELISA were tested using the second tier (IgG) Marblot western blot (MarDx Diagnosis, Trinity Biotech, Carlsbad, California) which was performed in accordance with the manufacturer's recommendation. The criteria used in the interpretation of the western blot as either negative or positive was based on the manufacturer's recommendation using established criteria (29).

Modified two-tiered testing:

The *Borrelia* VlsE1/pepC10 IgM/IgG test system (ZEUS Scientific, Branchburg, NJ) was performed on 91 C6 ELISA positive/equivocal samples as well as nine randomly chosen negative samples. All positive and equivocal samples were then tested by the *B. burgdorferi* ELISA whole cell antigen IgG test system (ZEUS Scientific) (30). Both ELISAs were performed in accordance with the manufacturer's recommendation (31).

GlpQ ELISA:

Borrelia miyamotoi recombinant his-tagged GlpQ antigen (1 µg/well) was bound to 96 well plates as described (24) with the following modifications. Peroxidase conjugated goat anti-human IgA+IgG+IgM (H&L) (1:2500) and SureBlue TMB peroxidase substrate (SeraCare, Milford, Massachusetts) were utilized to detect bound antibody. The positive cutoff was set at

three standard deviations above the mean absorbance of sera from four negative controls (healthy persons). A positive control from a *B. miyamotoi* PCR positive patient was included in each run. Absorbance was read at 450 nm.

B. miyamotoi **western blot:**

The *B. miyamotoi* western blot strips were produced using *B. miyamotoi* strain CT13-2396, which was originally isolated from *I. scapularis* collected in Connecticut; NCBI accession number: PRJNA310783. Strain CT13-2396 was grown in BSK-R medium and harvested by centrifuging at 10,000(g) for 10 minutes at 4 °C. The resulting cell pellet was frozen, thawed, and re-suspended in TE buffer (Fisher Scientific, Pittsburgh, PA), sonicated, and diluted to a final protein concentration of 2.0 mg/ml. The sonicate was mixed with sample buffer with DTT (Bio-Rad, Inverness, CA), heated at 95 °C heat block for 10 min, resolved on 12.5% SDS-PAGE gels for 180 minutes at 70mAmps, and soaked in tris/glycine buffer (Bio-Rad) with 20% methanol for 30 minutes at 4 °C. Separated proteins were transferred for 30 minutes at 25 volts to a 0.2 μm nitrocellulose membrane using a Trans-Blot® SD Semi-Dry Transfer Cell (Bio-Rad). Membranes were soaked overnight in 1% milk (Bio-Rad) and tris-buffered saline and Tween™ 20 (TBST)(Fisher Scientific), dried and then cut into 3 mm strips and stored at 4^{o}C. To perform western blotting, strips were re-hydrated in 1% milk and TBST (blocking buffer), then incubated with sera at a final concentration of 1:200 in blocking buffer for 30 min. Strips were then washed and incubated for 15 min in blocking buffer and phosphatase-labeled goat anti human IgG (H+ L) conjugate (KPL, Gaithersburg, MD) added at a concentration of 1:10,000, followed by a final wash series. Strips were developed using BCIP/NBT phosphatase substrate (KPL, Gaithersburg, MD). A control strip using a monoclonal GlpQ antibody was included as a locator for the GlpQ

antigen; it was processed identically, with the exception of using anti mouse IgG (H+ L) conjugate. *B. miyamotoi* and *B. burgdorferi* human sera were included as positive and negative controls, respectively.

Statistical analysis:

Statistical analysis of *B. burgdorferi* and *B. miyamotoi* results were performed separately. The outcome variable for each analysis was the serum sample as positive, equivocal, or negative for the pathogen. The exposure variable was blood donor's residence in high or low Lyme disease endemic counties, as defined above. Point prevalence (seroprevalence) was calculated as the number of people who tested positive for the pathogen over total number of people tested. Exact 95% confidence intervals were constructed for prevalence estimates. Sample t-tests and chi-square tests were used to compare characteristics of those providing samples between the high and low-endemic counties in the overall sample and those samples carried forward to confirmatory testing. A two-tailed Fisher's exact test was performed to compare the percentage of *Borrelia* species–seropositive study participants in the high and low endemic counties. A P value less than 0.05 was considered statistically significant. All analyses were performed using SAS software version 9.4, SAS Institute Inc., Cary, NC, USA.

Results:

Sample characteristics:

There was no significant difference in the distribution of males to females by risk level for the study population. Participants in low Lyme disease endemic counties were on average slightly younger than those in high Lyme disease endemic counties (41 years and 49 years, respectively

P<0.001). The majority of participants listed ethnicity as White and non-Hispanic, with a greater percentage of Hispanics in the low endemic counties (20.5%) than in the high endemic counties (6.7%; P<0.001) (Table 3.1). In the high endemic counties, over two-thirds of the samples were from San Mateo (38.0%) or Sonoma (20.6%) counties, while most of the samples from the low endemic counties were from Ventura (56.1%) and San Luis Obispo (31.6%) counties (Table 3.2).

Table 3.1: Demographic characteristics of sera samples and positive Bb STTT and Bm GlpQ

	All Sera Samples		P-Value	Positive *B. burgdorferi* Standard Two-Tiered Testing** (C6 ELISA + Marblot WB)		Positive *Borrelia miyamotoi* GlpQ** (GlpQ ELISA + Bm WB)	
	High Endemic (N=941)	Low Endemic (N=759)		High Endemic	Low Endemic	High Endemic	Low Endemic
Sex							
Male	521 (55.4%)	420 (55.3%)	0.7	7 (0.74%)	0 (0.00%)	0 (0.00%)	0 (0.00%)
Female	413 (43.9%)	346 (45.6%)		0 (0.00%)	1 (0.13%)	1 (0.11%)	1 (0.13%)
Age							
Mean Age (SD*)	49.0 (16.9)	41.1 (19.7)	<	65.3 yrs. (6.5)	63 yrs. (N/A)	17 yrs. (N/A)	32 yrs. (N/A)
Range	16yrs to 84yrs	16yrs to 84yrs	**0.001**	56 yrs. - 75 yrs.	N/A	N/A	N/A
Ethnicity							
Non-Hispanic	878 (93.3%)	603 (79.4%)	<	7 (0.74%)	1 (0.13%)	1 (0.11%)	0 (0.00%)
Hispanic	63 (6.7%)	156 (20.5%)	**0.001**	0 (0.00%)	0 (0.00%)	0 (0.00%)	1 (0.13%)

* Standard deviation not calculated for (n=1)
** Percentages calculated out of all sera samples in high or low endemic areas

Table 3.2: Testing results of sera samples for *B. burgdorferi* and *B. miyamotoi* by county			
Counties where Sera was Sampled	**C6 Lyme ELISA/Screening Test****	**Bb Standard Two Tier Testing*****	***Borrelia miyamotoi* GlpQ*****
N	n (%*)	n (%*)	n (%*)
High Endemic Counties			
Marin (n = 157)	12 (7.64%)	2 (1.27%)	0 (0.00%)
Mendocino (n = 38)	3 (7.89%)	0 (0.00%)	1 (2.63%)
Napa (n = 108)	3 (2.78%)	1 (0.93%)	0 (0.00%)
San Mateo (n = 358)	19 (5.31%)	3 (0.84%)	0 (0.00%)
Santa Clara (n = 86)	3 (3.49%)	0 (0.00%)	0 (0.00%)
Sonoma (n = 194)	9 (4.64%)	1 (0.51%)	0 (0.00%)
Low Endemic Counties			
Orange (n = 34)	0 (0.00%)	0 (0.00%)	0 (0.00%)
San Luis Obispo (n = 240)	16 (6.67%)	1 (0.42%)	0 (0.00%)
Solano (n = 59)	3 (5.08%)	0 (0.00%)	0 (0.00%)
Ventura (n = 426)	23 (5.40%)	0 (0.00%)	1 (0.23%)

* County percent is based upon the number of samples tested from each county.
** County percent include both positive and equivocal results
***County percent include only positive test results

C6 ELISA screen:

Of the 1,700 serum samples screened with C6 ELISA, 64 (3.76%) were positive and 27 (1.59%) were equivocal. Of the 941 samples from high endemic counties, 49 (5.2%) total samples were positive (n=38) or equivocal (n=11) for *B. burgdorferi* antibody compared to 42 (5.5 %; 26 positive and 16 equivocal) of the 759 samples from low endemic counties.

Ninety-one samples that were C6 ELISA positive/equivocal and nine randomly selected negative samples had additional testing. A little more than half (58%) of the 91 samples that were positive or equivocal were from male individuals. The mean age for males was 42.6 years (range 16 years to 80 years) while the mean age of females was 49.6 years (range 16 years to 77 years). Most individuals with positive or equivocal samples were non-Hispanic (87%). Ethnicity (high endemic: non-Hispanic (93.9%); low endemic: non-Hispanic (78.6%); P-value = 0.058), sex (high endemic: male 55.1%; low endemic: male 61.9%; P-value = 0.5) and age (high endemic:

mean = 49 years, SD = 16.6; low endemic: mean = 42 years, SD = 20.9; P-value = 0.07), of those with positive or equivocal samples did not differ between high and low endemic counties.

Of the nine randomly selected negative samples, two thirds were from female subjects. The mean age was 40.6 years for males (range 16 years to 72 years) and 35.7 years (range 25 years to 60 years) for females. Seven of the nine (78%) were non-Hispanic. Four of the nine (44%) selected negative of the samples were from high endemic counties while 5 of the 9 (56%) of samples were from low endemic counties.

***B. miyamotoi* GlpQ seroreactivity:**

Two of the 1,700 samples had detectable antibodies against *B. miyamotoi* (0.12%, Exact 95% CI: 0.01%, 0.42%). Both samples tested positive by C6 ELISA, GlpQ ELISA and *B. miyamotoi* whole cell western blot. Both samples were negative on the IgG western blot for *B. burgdorferi* (Figure 3.3, Table 3.3). None of the nine randomly selected C6 seronegative samples were seropositive for antibodies against *B. miyamotoi* by the GlpQ ELISA. Seroprevalence was (2.63%, Exact 95% CI: 0.7, 13.81) among Mendocino County residents and (0.23%, Exact 95% CI: 0.01, 1.30) among Ventura County residents. Both seropositive samples were from females with respective ages of 17 years and 32 years (Table 3.1).

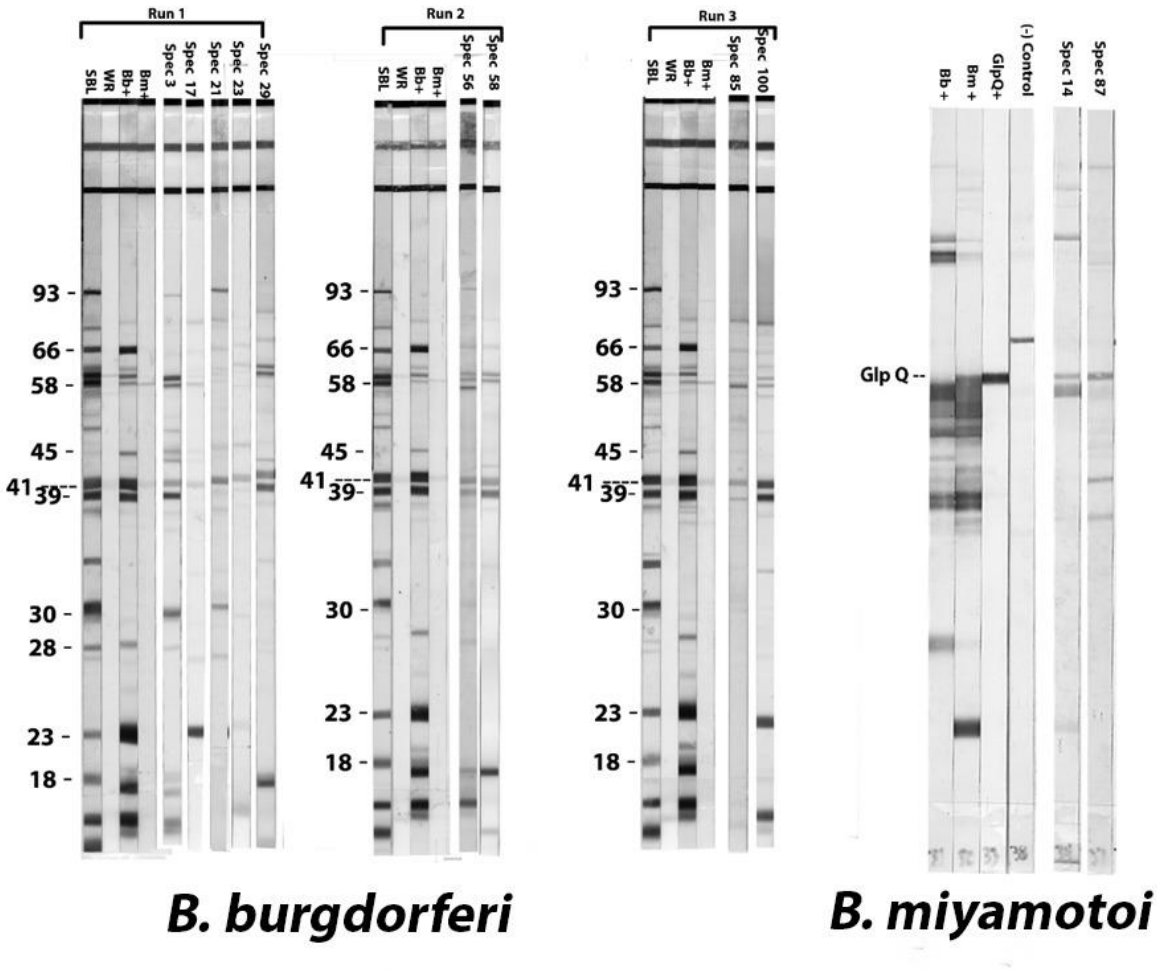

Figure 3.3: ***B. burgdorferi*** **and** ***B. miyamotoi*** **western blot seroreactivity in blood donors from higher and lower risk counties for Lyme disease in California:** For B. burgdorferi, each run included a serum band locator (SBL) control, which shows reactivity to Lyme diagnostically significant bands at position 93, 66, 58, 45, 41, 39, 30, 28, 23 and 18 kDa. Bands were scored relative to the intensity of the 41kDa band in the and weakly reactive (WR) control. Additionally, control serum from persons confirmed positive for B. burgdorferi or B. miyamotoi were included. Serum samples seropositive for 5 or more bands are shown. For B. miyamotoi western blots, control sera from patients confirmed positive for B. burgdorferi or B. miyamotoi were included along with a negative control serum. A monoclonal antibody to GlpQ was also included as a locator for the GlpQ antigen. The two samples with seroreactivity to GlpQ are shown.

B. burgdorferi **IgG western blot:**

Eight of 1,700 samples had detectable antibodies against *B. burgdorferi* (0.47%, Exact 95% CI:

0.20, 0.93). Of the eight sera samples that were positive by the C6 ELISA and *B. burgdorferi*

IgG western blot (STTT), seven (0.74%, Exact 95% CI: 0.30, 1.53) were from high Lyme

disease endemic counties and one positive sample (0.13%, Exact 95% CI: 0.00, 0.73) was from a low Lyme disease endemic county (P=0.08) (Table 3.1). Marin County had an overall seroprevalence of 1.3% (Exact 95% CI: 0.15, 4.53), Napa County had an overall seroprevalence of 0.93% (Exact 95% CI: 0.02, 5.05), San Mateo County had an overall seroprevalence of 0.84% (Exact 95% CI: 0.17, 2.43), and Sonoma County had an overall seroprevalence of 0.52% (Exact 95% CI: 0.01, 2.84). San Luis Obispo had an overall seroprevalence (0.4%) (Exact 95% CI: 0.01, 2.30) (Table 3.2).

Modified two-tiered testing:

Eight of the 91 C6 ELISA positive/equivocal sera were positive by modified two-tiered testing (MTTT) (Table 3.3). Six of the 91 C6 ELISA positive/equivocal sera positive by both STTT and MTTT were from a high endemic county and two of 91 C6 ELISA positive/equivocal sera positive by MTTT only were from a low endemic county. There was little agreement in STTT and MTTT positivity among C6 ELISA positive/equivocal sera from a low endemic county. Seven of the 91 C6 ELISA positive/equivocal sera positive by MTTT were male with mean age of 65 years (range 56 years to 75 years) and the age of the female was 16 years.

Table 3.3: Antibody testing results of human samples

Study Subject	*B. burgdorferi* (Standard Two-Tier Testing)					*B. burgdorferi* (Modified Two Tier Testing)					*B. miyamotoi* (GlpQ Testing)			
Sample Number	**C6 ELISA**		**Marblot WB (IgG)***		**Interpretation**	**Zeus vslE1/pepC10**		**Zeus whole cell IgG**		**Interpretation**	**GlpQ ELISA**		***B..miyamotoi* Western Blot**	
	Index Value	**Results**	**Pos Band**	**Results**		**Index Value**	**Results**	**Index Value**	**Results**		**OD value**	**> pos cutoff**	**GlpQ Band**	**Result**
High Endemic Counties														
Sample 3	**6.660**	**Positive**	**7**	**Positive**	**Positive**	**4.468**	**Positive**	**3.362**	**Positive**	**Positive**	1.210	2.799	No	Negative
Sample 17	**1.926**	**Positive**	**5**	**Positive**	**Positive**	0.269	Negative	N/A	N/A	Negative	1.471	2.799	No	Negative
Sample 21	**4.510**	**Positive**	**6**	**Positive**	**Positive**	**2.337**	**Positive**	**1.428**	**Positive**	**Positive**	1.278	2.799	No	Negative
Sample 29	**1.468**	**Positive**	**5**	**Positive**	**Positive**	**1.448**	**Positive**	**2.920**	**Positive**	**Positive**	0.953	2.145	No	Negative
Sample 56	**3.783**	**Positive**	**6**	**Positive**	**Positive**	**1.295**	**Positive**	**2.799**	**Positive**	**Positive**	0.589	2.234	No	Negative
Sample 58	**1.156**	**Positive**	**5**	**Positive**	**Positive**	**0.991**	**Equivocal**	**2.083**	**Positive**	**Positive**	0.622	2.234	No	Negative
Sample 87	**1.190**	**Positive**	4	Negative	Negative	0.254	Negative	N/A	N/A	Negative	**2.493**	**2.246**	**Yes**	**Positive**
Sample 100	**1.082**	**Equivocal**	**5**	**Positive**	**Positive**	**1.366**	**Positive**	**2.365**	**Positive**	**Positive**	1.145	2.246	No	Negative
Low Endemic Counties														
Sample 14	**0.990**	**Equivocal**	2	Negative	Negative	0.675	Negative	N/A	N/A	Negative	**3.094**	**2.799**	**Yes**	**Positive**
Sample 23	**0.957**	**Equivocal**	**6**	**Positive**	**Positive**	0.327	Negative	N/A	N/A	Negative	2.015	2.799	No	Negative
Sample 59	**3.540**	**Positive**	2	Negative	Negative	**1.604**	**Positive**	**0.965**	**Equivocal**	**Positive**	1.591	2.234	No	Negative
Sample 79	**>7.00**	**Positive**	3	Negative	Negative	**6.437**	**Positive**	**2.518**	**Positive**	**Positive**	0.360	1.666	No	Negative
Sample 85	0.220	Negative	**5**	**Positive**	Negative	0.225	Negative	N/A	N/A	Negative	0.865	1.666	No	Negative

* Marblot Western Blot - A positive sample is $\geq$ 5 bands out of 10 bands (All results were visually read)

Discussion:

This study represents the largest serosurvey across a broad geographic area in California estimating human exposure to *B. miyamotoi* and *B. burgdorferi*. We found higher *B. burgdorferi* antibody estimates from higher risk Lyme disease endemic areas in northern California but similar *B. miyamotoi* antibody estimates from high and low risk Lyme disease endemic areas in the state. Our large sample size of 1,700 and broad geographic expanse increases the confidence of our seroprevalence estimate. Although the overall risk of human acquisition of either pathogen is lower in California compared with those in high-risk Lyme disease endemic areas in the Northeast and northern Midwest, endemic areas are shifting with climate and human habitat changes that alter the epidemiology of these infections (32-34). Our data align with previous research that demonstrated the C6 Lyme ELISA test can detect seroreactivity to both *B. burgdorferi* and *B. miyamotoi* because the C6 peptide sequence in the C6 ELISA test kit found in *B. burgdorferi* is very similar to the relapsing fever *Borrelia* variable large protein (Vlp) sequence, including Vlp 15/16 of *B. miyamotoi* (23, 35). The cross-reactive antibodies against the C6 peptide occurs in 90% of patients with *B. miyamotoi* disease (35). The C6 ELISA test can also detect antibodies to all of the major pathogenic European *Borrelia* species: *B. afzelii*, *B. garinii*, and *B. burgdorferi* (36).

Only two of the 1,700 serum samples were positive for both C6 and GlpQ antibodies (0.12%, Exact 95% CI: 0.01, 0.42]). This result suggests that these two persons had prior *B. miyamotoi* infection, given that the *glpQ* gene is not present in Lyme spirochetes (23, 24). With so few *B. miyamotoi* seropositive samples, we had insufficient power to detect a difference between high and low Lyme disease endemic counties. Overall, in California, the risk of exposure to *B.*

miyamotoi is relatively low. However, there may be ecologic foci of exposure risk, similar to what is seen for Lyme disease in California (2). For example, the *B. miyamotoi* seroprevalence in Mendocino County (2.6%) is similar to that noted in a previous study (21) which documented seroprevalence values of 1.98% and 6.93% over several years in a Mendocino community. The population studied was at high risk of tick-borne disease because of well-documented *I. pacificus* tick exposure (21).

There is a potential for cross reactivity to the *B. miyamotoi* GlpQ antigen in persons with prior exposure to *B. hermsii* (37, 38). *Borrelia hermsii* is the primary etiologic agent of tick-borne relapsing fever (TBRF) in the western United States and transmitted by the argasid (soft) tick *Ornithodoros hermsi* (39, 40) with about 2 to 20 cases per year in California (41). The primary hosts for these ticks are rodents (39, 40). The range of the vector that carries *B. hermsii* are typically found at higher elevations (914 meters to 2743 meters) from the southern Cascades to the Eastern Sierra Nevada Mountain range down to the Southern California Mountains (39). Ventura County's proximity to *B. hermsii* endemic areas (within 120 km), and the fact that no *I. pacificus* ticks have tested positive for *B. miyamotoi* from Ventura County (16, 41) suggest that we cannot rule out that the *B. miyamotoi* seropositive sample from Ventura County represents seropositivity to *B. hermsii*. Mendocino County, by contrast, has had *I. pacificus* ticks with documented *B. miyamotoi* infection and is geographically distant from *O. hermsii* distribution (21), supporting the *glpQ* -positive sample more likely to represent *B. miyamotoi* exposure. Both GlpQ positive samples for *B. miyamotoi*, not only can represent cross reactivity with *B. hermsii*, but the infections may have been acquired outside the county or state. Although *B. miyamotoi*

has a low seroprevalence in California, our study findings are consistent with a previous study that humans in California are exposed to *B. miyamotoi* (21)

The overall seroprevalence for *B. burgdorferi* from both high and low Lyme disease endemic counties in California for Lyme disease was 0.47% (Exact 95% CI: 0.20, 0.93). The STTT, utilizing the C6 ELISA and IgG western blot, is more stringent and specific then the IgM western blot (42). Since blood bank donors are generally healthier than the general population (43, 44) and the incidence of Lyme disease in California is about 0.2 cases per 100,000 population (16), a *B. burgdorferi* seroprevalence of less than 0.5% in California appears to be a reasonable estimate.

For California, a local approach to estimating risk is important for public health communication, given the well-documented non-uniform exposure due to local ecological influences (2, 11, 17, 45). Few studies are available from California measuring *B. burgdorferi* seroprevalence in specific communities. In one study in Sonoma County, 1.4% of a small community were found to be seropositive for *B. burgdorferi*, an estimate within our confidence level estimates for that county, while in the same study, no samples from a blood bank collection from Sacramento County tested positive (46). Although we did not find a statistically significant difference in seroprevalence between high and low Lyme disease endemic counties, all but one of our *B. burgdorferi* positive samples came from high endemic counties. Our study provides an updated estimate of *B. burgdorferi* exposure in a broad geographic area of California and helps demonstrate that risk for Lyme disease is geographically diverse. A more recent study found that 3.2% of residents in high endemic counties for Lyme disease in northern California may have

been exposed to *B. burgdorferi* (47) as measured by the C6 ELISA alone. This estimate is within our confidence level estimates for C6 positive/equivocal samples. However, it is important that our C6 positive or equivocal samples were followed by testing with a more specific IgG Lyme western blot assay to avoid over estimation of *B. burgdorferi* exposure and to differentiate *B. miyamotoi* exposure. The increased sensitivity and decreased specificity of the C6 ELISA could over-estimate true exposure if used as a stand-alone test for estimating seropositivity, particularly in low incident states (48, 49) and should be used in conjunction with second tier assays in the recommended STTT format for Lyme disease diagnosis (50, 51).

The MTTT is intended for the qualitative detection of antibodies to *B. burgdorferi* in human serum (31). With the advent of both the VslE1 and peptide C10 in the first tier ELISA assay, the MTTT assay we utilized is designed to be both sensitive and specific for early and late infection in patients with Lyme disease (31, 52). The C6 peptide derived from VlsE1 does not bind to IgM well, and therefore the C6 ELISA is not ideal for detecting early cases of Lyme disease (48, 53). On the other hand, an IgM response is generated early in disease in response to the pepC10 protein (52). Among the 91 samples seropositive or equivocal by the C6 ELISA, the overall seropositivity was the same for MTTT and STTT. However, minor discrepancies were noted: Samples 59 and 79 were negative by STTT but positive by MTTT and Samples 17 and 23 were positive by STTT but negative by MTTT. Samples 59 and 79 had high Lyme index values by C6 ELISA but were negative on the Lyme disease western blot (IgG), possibly indicating that both samples were taken early after infection, or the person had been treated early after illness onset and did not produce an expanded IgG response detectable by the Lyme disease western blot criteria. In contrast, samples 17 and 23 had lower index values by C6 ELISA and were

positive by western blot, possibly indicating an old infection, with waning pepC10 antibody response that could not be picked up by the MTTT.

There are some limitations to this study. The necessary deidentified nature of human serum from blood bank samples precludes analysis of potential risk factors such as travel to *B. burgdorferi*, *B. miyamotoi* or *B. hermsii* endemic areas or degree of tick exposure. Human antibodies to the C6 peptide for *B. burgdorferi* wane after two years following untreated *B. burgdorferi* infection and probably more rapidly if treated (54). The sensitivity of C6 for detecting *B. miyamotoi* infection and the longevity of the C6 antibody response in *B. miyamotoi* infection is also not known. Human IgG antibody response dynamics to *B. miyamotoi* are still unknown (55). Lack of exposure information decreases the overall prior probability of having the disease in relation to diagnostic testing. Only nine negative samples were chosen due to resource limitations of second tier testing. Though the samples could have been matched by gender, given the small number of negative samples available, the authors chose to balance by endemicity status. Since there was no difference in seropositivity between males and females, we felt that the 2/3 bias towards females in the negative samples did not compromise the study. Sampling sera from blood donors may decrease the generalizability of the results to the general population (56).

Conclusions:

Our study demonstrates that California residents are at risk for infection by the emerging *I. pacificus*-transmitted pathogens *B. miyamotoi* and *B. burgdorferi*, although they are at relatively low risk of these infections even in the most highly endemic counties for Lyme disease. Standard two-tier *B. burgdorferi* testing and GlpQ serologic testing for *B. miyamotoi* on C6 positive or

equivocal samples determined specific reactivities to these agents. Among samples testing C6 positive/equivocal, the STTT and MTTT performed fairly consistently even in this population with no known exposure history and in a state with low endemicity for Lyme disease. Further investigation of risk mapping related to geography and habitat type are needed for *B. miyamotoi*.

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Conclusion:

This dissertation is to evaluate the current understanding of hard tick associated *Borrelia* infections in California, specifically *Borrelia burgdorferi* and *Borrelia miyamotoi* in California by focusing on these three main objectives: [1] To evaluate whether estimation procedures through predictive modeling can reduce the information necessary to classify a case in order to improve case reporting at local level; [2] to assess an under-studied area of physician knowledge and practice of testing and treatment of Lyme disease in a low incidence state; and [3] to determine whether *B. miyamotoi* exposure occurs in high endemic areas for Lyme disease and to assess if *B. burgdorferi* and *B. miyamotoi* have the same geographic range since both pathogens share the same tick vector.

California has seen a marked increase of case reports for Lyme disease due to the advent of electronic case and laboratory reporting of reportable infectious diseases in 2011. However, the number of reportable cases of Lyme disease remained fairly stable, despite the increase in case reports. An increased number of incomplete case reports that require follow-up may overburden local health departments who must investigate all reported positive lab reports to gather the necessary clinical information to classify a case of Lyme disease. Due to a variety of reasons, including the amount of follow up information necessary, and to the complexity of the Lyme disease case definition, many cases are not followed up adequately to obtain all relevant information necessary to classify a case. High-incidence states for Lyme disease, such as New York, New Jersey, and Massachusetts have used estimation sampling approaches to approximate the incidence of Lyme disease in their respective states. Estimation sampling procedures have shown to yield a good approximation of Lyme disease in these high incidence states, thus we

assessed whether estimation procedures would work to approximate the incidence of Lyme disease in California, which is a low incidence state with a large human population.

We used predictive modeling, starting with a simple model with only one predictor (positive IgG western blot) and created three other models building upon each other. Two of the models included information that is automatically reported to CalREDIE that required no further investigation. The last two models built upon the second model but added one piece of information that would require further investigation. Surveillance data obtained from CalREDIE is the standard we used to compare our predicted models against as it is the most complete source of data estimating Lyme disease incidence in California. Each of the four predictive models had very low sensitivities and the results showed that the four predictive models based on subsets of contextual, vector, and diagnostic testing data would underestimate the incidence of Lyme disease in California. Our results showed that such estimation procedures would not result in an accurate approximation of the incidence of Lyme disease in California. We anticipate that the results from this research can inform state health department in low incidence states, such as the Californian Department of Public Health, on strategies to enhance surveillance practices for Lyme disease. Further research is needed to evaluate the balance of effort to collect necessary information to classify a case report consistently across all health jurisdictions with the return of pertinent epidemiologic information that meets the primary goals of surveillance.

The clinical diagnosis of Lyme disease is based upon understanding and recognition of signs and symptoms in patients, laboratory results, and patient exposure histories. Effective diagnostic approaches depend on the physician's knowledge and awareness of Lyme disease in the

community in which they practice. These complexities, coupled with exposure in a low incidence state, such as California, can make the diagnosing of Lyme disease more complicated. Physician assessment in high incidence states for Lyme disease suggested physicians generally are knowledgeable and followed the national published guidelines for the diagnosis and treatment of symptomatic Lyme disease but were likely to deviate from guidelines for asymptomatic tick bites.

We distributed surveys among California physicians to determine if knowledge and practice of testing and treating differed between physicians practicing in high endemic areas compared to physicians practicing in low endemic areas in an overall low incidence state for Lyme disease. Only 62 physicians responded to our survey as this study was conducted at the height of the global pandemic. While our low response rate precluded us from detecting significant differences between high and low endemic areas, by focusing on care type in each area, we were able to identify some interesting patterns in knowledge of testing and treating patients in the context of Lyme disease. To the extent that our surveyed physicians represent California physicians, physicians in California could benefit from further targeted education to better understand disease risk in California and to improve recognition of symptoms and appropriate use and interpretation of serologic testing.

Other potentially zoonotic spirochetes have been documented in the western blacklegged tick (*Ixodes pacificus*) which includes *B. miyamotoi*, an emerging tick-borne pathogen that is in same genus as the agents of relapsing fever *Borrelia*. *Borrelia miyamotoi* was first discovered in Japan in 1994, with a human case reported in 2011 from Russia. Since then, human cases have been

reported in Europe, Japan, and eastern and midwestern United States. Infection with *B. miyamotoi* causes a relapsing fever like illness, also known as *Borrelia miyamotoi* disease.

In California, previous studies have shown that the minimum infection prevalence (MIP) for adult *I. pacificus* ticks was 0.7 (range 0.3 – 10.0) and the MIP for nymphal *I. pacificus* ticks was 3.4 (range 0.9 – 50) for *B. burgdorferi*. The MIP for adult *I. pacificus* ticks was 0.8 (range 0.3 – 12.5) and the MIP for nymphal *I. pacificus* ticks was 1.1 (range 0.9 –4.6) for *B. miyamotoi.* The distribution of *B. miyamotoi* in *I. pacificus* ticks appears to be similar to that of *B. burgdorferi* and is most prevalent in coastal and foothill regions of northern California.

We assessed human exposure to *B. burgdorferi* and *B. miyamotoi* by testing 1,700 blood bank serum samples from both high and low Lyme disease endemic areas in California. Of the 1,700 samples, 941 samples were from high endemic counties and 759 samples were from low endemic counties for Lyme disease. All sera samples were screened using a C6 Lyme ELISA. Samples positive or equivocal by C6 Lyme ELISA were tested using the second tier (IgG) Marblot western blot (Standard Two-tier testing - STTT) and the new modified two-tier testing (MTTT) with the use of two ELISAs. Two of the 1,700 samples had detectable antibodies against *B. miyamotoi* (0.12%, Exact 95% CI: 0.01%, 0.42%). One of the positive samples was from Mendocino County; county seroprevalence was (2.63%, Exact 95% CI: 0.7, 13.81). The other positive sample was from Ventura County and county seroprevalence was (0.23%, Exact 95% CI: 0.01, 1.30). Both seropositive samples were from females with respective ages of 17 years and 32 years. Eight of 1,700 samples had detectable antibodies against *B. burgdorferi* (0.47%, Exact 95% CI: 0.20, 0.93). Of the counties where positive samples were identified,

Marin County had an overall seroprevalence of 1.3% (Exact 95% CI: 0.15, 4.53), Napa County had an overall seroprevalence of 0.93% (Exact 95% CI: 0.02, 5.05), San Mateo County had an overall seroprevalence of 0.84% (Exact 95% CI: 0.17, 2.43), and Sonoma County had an overall seroprevalence of 0.52% (Exact 95% CI: 0.01, 2.84). San Luis Obispo had an overall seroprevalence (0.4%) (Exact 95% CI: 0.01, 2.30).

Among our tested blood bank samples, we found higher *B. burgdorferi* antibody prevalence estimates from higher risk Lyme disease endemic areas in northern California but similar *B. miyamotoi* antibody estimates from high and low risk Lyme disease endemic areas in the state. This study demonstrated that California residents are at risk for infection by the emerging *I. pacificus*-transmitted pathogens such as *B. miyamotoi* and *B. burgdorferi*, although at relatively low risk even in the most highly endemic counties for Lyme disease. Our study also explored the performance of the MTTT in a low endemic state, and among samples testing C6 positive/equivocal, the STTT and MTTT performed fairly consistently.

Since the advent of mandatory reporting of all positive lab results in 2005 and the implementation of electronic lab reporting integrated into CalREDIE in 2011, California has not seen a huge increase in confirmed cases of Lyme disease. However, since the advent of these reporting tools, California has seen a significant increase in the number of suspect case reports submitted into CalREDIE. Our results showed that additional information such as clinical symptoms or travel history are necessary and overall improves both the sensitivity and specificity of California's surveillance data. Physician awareness and knowledge about Lyme disease remains a challenge. The inappropriate use of an IgM western blot can result in high

false positive rate which inexplicably remains a problem. The results of this dissertation can be immediately pertinent to public health practice, such as health education programs. We performed the first comprehensive look at *B. miyamotoi* in California since discovered in western blacklegged ticks in 2003. This research study on *B. miyamotoi* seroprevalence adds to the literature on exposure to this agent in California, but to date no clinical descriptions of *B. miyamotoi* disease acquired in California exist. The results of this dissertation taken together have provided important insights regarding *B. burgdorferi* (Lyme disease) and *B. miyamotoi* (*Borrelia miyamotoi* disease) in California.

Chapter 1 Appendix:

The following code was used to run the iterations of the 10 training and 10 testing sets for K-fold cross validation, where K=10. This code was setting up the macro.

```
%macro k_fold_cv(k=10);
ods select none;

%do i=1 %to &k ;
data training;
 set have(where=(groupid ne &i)) ;
run;
data test;
 set have(where=(groupid eq &i));
run;

*Model 1 and ROC;
ods output
Association=native1(keep=label2 nvalue2 rename=(nvalue2=native) where=(label2='c'))
ScoreFitStat=true1(keep=dataset freq auc rename=(auc=true));
proc logistic data=training
 outest=est1(keep=_status_ _name_);
 class posigg(ref="0")/param=ref;
 model rstatus(event='1')=posigg /outroc=troc;
score data=test out=mod1pred fitstat outroc=mod1vroc ; *maybe add out=modpred to get
predicted probabilities from test set?;
run;


data mod1vroc&i;
  set mod1vroc;
  run;

data mod1pred&i;
  set mod1pred;
  retain f_rstatus i_rstatus; * need predicted variable names;
run;

data score1_&i;
 merge true1 native1 est1;
 retain id &i ;
 optimism=native-true;
run;

*Model 2 and ROC;
ods output
Association=native2(keep=label2 nvalue2 rename=(nvalue2=native) where=(label2='c'))
```

```
ScoreFitStat=true2(keep=dataset freq auc rename=(auc=true));
proc logistic data=training
 outest=est2(keep=_status_ _name_) ;
 class posigg(ref="0") season(ref="winter") agecat(ref="2")/param=ref;
 model rstatus(event='1')=posigg agecat sex Adult_MIP season /outroc=troc;
 score data=test out=mod2pred fitstat outroc=mod2vroc; *maybe add out=modpred to get
predicted probabilities from test set?;
run;

data mod2vroc&i;
  set mod2vroc;
  run;

data mod2pred&i;
  set mod2pred;
  retain f_rstatus i_rstatus; * need predicted variable names;
run;

data score2_&i;
 merge true2 native2 est2;
 retain id &i ;
 optimism=native-true;
run;


*Model 3 and ROC (Clinical no Travel);
ods output
Association=native3(keep=label2 nvalue2 rename=(nvalue2=native) where=(label2='c'))
ScoreFitStat=true3(keep=dataset freq auc rename=(auc=true));
proc logistic data=training
 outest=est3(keep=_status_ _name_) ;
 class posigg(ref="0") season(ref="winter") agecat(ref="2")/param=ref;
 model rstatus(event='1')=posigg agecat sex Adult_MIP season SEM dessym / outroc=troc;
 score data=test out=mod3pred fitstat outroc=mod3vroc; *maybe add out=modpred to get
predicted probabilities from test set?;
run;

data mod3vroc&i;
  set mod3vroc;
  run;

data mod3pred&i;
  set mod3pred;
  retain f_rstatus i_rstatus; * need predicted variable names;
run;
```

```
data score3_&i;
 merge true3 native3 est3;
 retain id &i ;
 optimism=native-true;
run;

*Model 4 Travel No Clinical);
ods output
Association=native3b(keep=label2 nvalue2 rename=(nvalue2=native) where=(label2='c'))
ScoreFitStat=true3b(keep=dataset freq auc rename=(auc=true));
proc logistic data=training
 outest=est3b(keep=_status_ _name_) ;
 class posigg(ref="0") season(ref="winter") travel(ref="0") agecat(ref="2")/param=ref; *travel=0
low incidence;
 model rstatus(event='1')=posigg agecat sex Adult_MIP season travel / outroc=troc;
 score data=test out=mod3bpred fitstat outroc=mod3bvroc; *maybe add out=modpred to get
predicted probabilities from test set?;
run;

data mod3bvroc&i;
  set mod3bvroc;
  run;

data mod3bpred&i;
  set mod3bpred;
  retain f_rstatus i_rstatus; * need predicted variable names;
run;

data score3b_&i;
 merge true3b native3b est3b;
 retain id &i ;
 optimism=native-true;
run;

%end;

data k_fold_cv_score_mod1;
 set score1_1-score1_&k;
run;

data k_fold_cv_score_mod2;
 set score2_1-score2_&k;
run;

data k_fold_cv_score_mod3;
 set score3_1-score3_&k;
```

```
run;

data k_fold_cv_score_mod3b;
 set score3b_1-score3b_&k;
run;


ods select all;
%mend;

%k_fold_cv(k=10)


Proc logistic for each predictive model including the code to construct the ROC curve along with
the 95% CI.
*Model 1;
ods output
Association=native1(keep=label2 nvalue2 rename=(nvalue2=native) where=(label2='c'))
ScoreFitStat=true1(keep=dataset freq auc rename=(auc=true));
proc logistic data=training
 outest=est1(keep=_status_ _name_) ;
 class posigg(ref="0")/param=ref;
 model rstatus(event='1')=posigg;
 score data=test out=mod1pred fitstat; *maybe add out=modpred to get predicted probabilities
from test set?;
run;

*ROC Curve for Model 1;
proc logistic data=training
 outest=est1(keep=_status_ _name_) desc ;
 class posigg(ref="0")/param=ref;
 model rstatus=posigg /outroc=troc;
 score data=test out=mod1pred outroc=vroc;  *maybe add out=modpred to get predicted
probabilities from test set?;
roc; roccontrast;
run;

*95% CI for Test ROC curve Model 1;
proc logistic data=mod1pred;
	model rstatus(event="1")=;
	roc pred=P_1;
	roccontrast;
	run;

*Model 2;
ods output
```

```
Association=native2(keep=label2 nvalue2 rename=(nvalue2=native) where=(label2='c'))
ScoreFitStat=true2(keep=dataset freq auc rename=(auc=true));
proc logistic data=training
 outest=est2(keep=_status_ _name_);
 class posigg(ref="0") season (ref="winter") agecat(ref="2") sex(ref="1")/param=ref ;
 model rstatus(event="1")=posigg agecat sex Adult_MIP season;
 score data=test out=mod2pred fitstat; *maybe add out=modpred to get predicted probabilities
from test set?;
run;

*ROC Curve For Model 2;
proc logistic data=training
 outest=est2(keep=_status_ _name_);
 class posigg(ref="0") season (ref="winter") agecat(ref="2")/param=ref ;
 model rstatus(event="1")=posigg agecat sex Adult_MIP season/outroc=troc;
 score data=test out=mod2pred outroc=vrocout;*maybe add out=modpred to get predicted
probabilities from test set?;
 roc;
run;

*95% CI for Test ROC Curve Model 2;
proc logistic data=mod2pred;
      model rstatus(event="1")=;
      roc pred= p_1 ;
                  roccontrast;
      run;

*Model 3;
ods output
Association=native3(keep=label2 nvalue2 rename=(nvalue2=native) where=(label2='c'))
ScoreFitStat=true3(keep=dataset freq auc rename=(auc=true));
proc logistic data=training
 outest=est3(keep=_status_ _name_) ;
 class posigg(ref="0") season(ref="winter") agecat(ref="2") sex(ref="1")/param=ref ;
 model rstatus(event='1')=posigg agecat sex Adult_MIP season SEM dessym ;
 score data=test out=mod3pred fitstat; *maybe add out=modpred to get predicted probabilities
from test set?;
run;

*ROC Curve for Model 3;
proc logistic data=training
 outest=est3(keep=_status_ _name_) ;
 class posigg(ref="0") season(ref="winter") agecat(ref="6")/param=ref ;
 model rstatus(event='1')=posigg agecat sex Adult_MIP season SEM dessym /outroc=troc;
 score data=test out=mod3pred outroc=vrocout; *maybe add out=modpred to get predicted
probabilities from test set?;
```

```
 roc;
run;

*95% CI for Test ROC Curve Model 3;
proc logistic data=mod3pred;
      model rstatus(event="1")=;
           roc pred= p_1 ;
               roccontrast;
           run;

*Model 4;
ods output
Association=native3b(keep=label2 nvalue2 rename=(nvalue2=native) where=(label2='c'))
ScoreFitStat=true3b(keep=dataset freq auc rename=(auc=true));
proc logistic data=training
 outest=est3b(keep=_status_ _name_) ;
 class posigg(ref="0") season(ref="winter") travel(ref="0") agecat(ref="2")
sex(ref="1")/param=ref ; *travel=0 low incidence;
 model rstatus(event='1')=posigg agecat sex Adult_MIP season travel ;
 score data=test out=mod3bpred fitstat; *maybe add out=modpred to get predicted probabilities
from test set?;
run;

*ROC Curve Model 4;
proc logistic data=training
 outest=est3b(keep=_status_ _name_) ;
 class posigg(ref="0") season(ref="winter") travel(ref="0") agecat(ref="6"); *travel=0 low
incidence;
 model rstatus(event='1')=posigg agecat sex Adult_MIP season travel/outroc=troc  ;
 score data=test out=mod3Bpred outroc=vrocout; *maybe add out=modpred to get predicted
probabilities from test set?;
 roc;
run;

*95% CI for Test ROC Curve Model 4;
proc logistic data=mod3Bpred;
      model rstatus(event="1")=;
      roc pred= p_1 ;
                roccontrast;
      run;
```

Chapter 2 Appendix:

Physician Survey:

Please complete the survey below.

Thank you for volunteering 5 to 10 minutes of your time to complete this brief survey. We are conducting research to determine common clinical knowledge and laboratory testing practices for Lyme disease in California. Your participation is completely voluntary and anonymous. All responses to this survey will be analyzed in the aggregate. Your participation in this survey will improve surveillance methodologies and improve public health education programs. If you have any questions about this research project, please feel free to contact Sharon I Brummitt, MPH (PhD candidate at University of California at Davis) at Sibrummitt@ucdavis.edu. Thank you again for your time and participation.

(1) I am a…….. ☐ Physician
☐ Other
Please specify (Other)_____

(2) What is your main area of practice? (Select one) ☐ Internal Medicine
☐ Family Medicine
☐ Pediatrics
☐ Infectious Diseases
☐ Other
Please Specify (Other) _____

(3) What is your practice setting? ☐ Outpatient
☐ Hospital
☐ Combination Outpatient and Hospital
☐ Urgent Care
☐ Other
Please Specify (Other)_____

(4) How many years have you been in practice? _____

(5) In what California County is your practice located? _____

Lyme disease Practices

(1) Within your geographic area of practice, would you consider Lyme disease endemic?

☐ Yes
☐ No
☐ Not Sure

(2) Have the number of Lyme disease cases increased among patients in your practice?

☐ Yes
☐ No
☐ Not Sure

(3) If you submitted a tick recovered from a patient for identification, would knowing the tick species inform your medical decision-making about Lyme disease?

☐ Yes
☐ No
☐ Not Sure

Please feel free to comment? _____

(4) If you submitted a tick recovered from a patient to be tested for *Borrelia burgdorferi*, would the tick testing result inform your medical decision-making about Lyme disease?

☐ Yes
☐ No
☐ Not Sure

Please feel free to comment? _____

(5) What Lyme disease diagnostic tests do you commonly order for a suspected Lyme disease patient? (Choose all that apply)

☐ Western Blot (IgG)
☐ EIA/IFA/ELISA
☐ PCR (Blood, Tissue)
☐ Culture
☐ CD57
☐ Western Blot (IgM)
☐ PCR (Synovial Fluid)
☐ Plasmid
☐ Other

Other (Please describe) _____

(6) In the past have patients asked you to be treated for Lyme disease though you have explained that Lyme disease was the unlikely cause of their symptoms?

☐ Never
☐ Rarely
☐ Occasionally
☐ Often

Please feel free to comment? _____

Patient Scenarios

The purpose of these questions is to assess common practices, not to test right or wrong. Please answer as you would practice on a typical day.

(1) A healthy patient with a history of daily hiking in the month of April and in an area where ticks are found, presents in your office with a rash resembling an erythema migrans that began 3 days earlier. You order a serologic test for Lyme disease which yields a negative result. Would you consider this negative test result definitive to rule out Lyme disease as the cause of this patient's rash?

☐ Yes
☐ No
☐ Maybe (Please comment)

Maybe (Please comment) _____

(2) A 50-year-old patient from Northwest California presents with a swollen, erythematous knee for the past week. The patient does not remember a tick bite or rash but is active outdoors and went on a hiking trip to the coastal foothills two months ago. You suspect Lyme disease. Which of the following testing approaches would yield the most diagnostic information?

☐ No Testing needed, treat the patient with appropriate antibiotic for Lyme disease
☐ Order an EIA only
☐ Order a Western blot only
☐ Order an EIA test followed by a reflex Western blot if EIA is positive
☐ Aspirate the joint and order PCR of the fluid
☐ Other (Please describe)

Other (Please describe) _____

(3) A 45-year-old patient from Southern California presents with fatigue and difficulty concentrating for the past two years. The patient does not remember a tick bite or rash but occasionally gardens in the backyard. The patient has not travelled out of Southern California for the past two years. A Lyme disease test was ordered at the time of the visit and the results were: Equivocal EIA, positive IgM Western blot (2/3 bands), negative IgG Western blot (1/10 bands). What is your interpretation of these results?

☐ The patient is unlikely to have Lyme disease
☐ The patient is likely to have Lyme disease
☐ Other (Please describe)

Other (Please describe) _____

(4) How would you interpret this test result from a patient you tested for Lyme disease: Negative EIA, positive IgM western blot, and negative IgG western blot?

☐ The patient is unlikely to have Lyme disease
☐ The patient is likely to have Lyme disease
☐ Other (Please describe)

Other (Please describe) _____

(5) A 35 year old patient was diagnosed (based upon positive serology and compatible clinical symptoms) and treated for Lyme disease. Are additional serologic test for Lyme disease warranted after treatment?

☐ Yes
☐ No
☐ Not Sure

Please feel free to comment? _____

(6) A patient presents to your clinic concerned with a tick bite received about 30 days ago. The patient has not travelled outside of CA, the patient has no symptoms, no laboratory testing performed to date, and normal examination findings. Which of the following describes your next action?

☐ Treat for Lyme disease with appropriate antibiotic at this time
☐ Treat the tick bite prophylactically with appropriate antibiotic at this time
☐ Do not treat for Lyme disease at this time
☐ Other (describe)

Other (Please describe) _____

(7) A patient presents with recurrent, asymmetric arthritis that began 3 months prior, involving large, weight-bearing joints. The patient has no history of an erythema migrans rash and has had multiple negative Western (IgM/IgG) blot test results for Lyme disease over the past 3 months. The patient does not recall a tick bit, but the patient spends a lot of time outdoors. Which of the following describes your next action?

☐ Treat for Lyme disease with appropriate antibiotic at this time
☐ Do not treat for Lyme disease at this time
☐ Other (describe)

Other (Please describe) _____

Resources

What common materials or websites do you refer your patients for Lyme disease information?

What tools or information would you find helpful in your practice to recognize, diagnose, or communicate about Lyme disease to your patients?

Lyme disease flyer:

Lyme disease flyer to help with the distribution of the physician survey.

Let's think about Lyme disease!

Tick season in California is now!!

Help me help you provide the best information to your patients about Lyme disease.

Please take this survey by August 1, 2020:
https://redcap.ucdmc.ucdavis.edu/redcap/surveys/?s=D7FJAPWAWH

Questions? Feel free to contact me:
Sharon Brummitt, MPH, PhD Candidate Epidemiology: UC Davis
Sibrummitt@ucdavis.edu

The study was determined to have exempt status by the Institutional Review Board, University of California Davis (Protocol Number 1090480-1)

Lyme disease article:

Is Lyme disease a risk to people during the COVID 19 pandemic?

By: Sharon I Brummitt (PhD Candidate in Epidemiology at UC Davis)

As COVID 19 rages throughout our world, so are other diseases that are not on everyone's radar. Vector-borne diseases account for more than 17% of all infectious diseases causing more than 700,000 deaths annually worldwide (WHO, March 2020: https://www.who.int/news-room/fact-sheets/detail/vector-borne-diseases). Recently, vector-borne diseases are making front page news, for example, Southeast Asia are in the midst of battling the Coronavirus while experiencing exploding numbers of dengue fever cases. A New Hampshire adult was recently diagnosed with James River Canyon Virus, its first case of their season, and researchers forecasting a bad season for Lyme disease. A recent headline on July 7, 2020, stated that "*Ticks and Lyme disease might be more common this year during the coronavirus pandemic*", so is the risk of Lyme disease higher during a pandemic and why? The Lyme disease research group from the University of New Haven, is concerned that the mild winter could lead to an increase in ticks carrying Lyme disease. Since the shelter in place orders have been lifted, we have seen an increase of hiking nationwide. Together, with an increase of infected ticks and an increase of hikers could ultimately lead to increase of infected individuals with Lyme disease.

Lyme disease is the most common tick-borne disease reported in the United States, with over 30,000 cases reported annually nationwide and roughly 100 cases reported annually in California. Although, California is classified as a low incidence state for Lyme disease with approximately 100 reported cases annually (CDPH, 2005-2013), however incidence of Lyme disease varies in California with higher risk areas (3.0 – 6.0 cases/100,000 population) in

northwest coastal counties and northern counties with western-facing Sierra Nevada slopes. My research suggests that awareness about tick-borne diseases in CA (whether endemic or travel related) is not as high as it could be. We need your help in understanding Lyme disease in California.

Please participate in taking this survey:
https://redcap.ucdmc.ucdavis.edu/redcap/surveys/?s=D7FJAPWAWH.

Chapter 3 Appendix:

The following table reflects the essential bands on the IgG western blot (*Borrelia burgdorferi*) for confirmation of a case and positive by the GlpQ western blot (*Borrelia miyamotoi*). According to the CDC 5 of 10 essential bands need to be positive to have a positive IgG western blot. This table shows participants that had a positive IgG western blot.

	Essential Bands for Western Blot (*Borrelia burgdorferi*)										**B. miyamotoi GlpQ band**
Sample Number	Band 93	Band 66	Band 58	Band 45	Band 41	Band 39	Band 30	Band 28	Band 23	Band 18	Glp Q
Sample 3	P	W	P	P	P	P	P	N	N	P	N
Sample 14*	N	P	N	N	P	N	N	N	N	N	P
Sample 17	N	P	P	W	P	N	N	P	P	N	N
Sample 21	P	P	P	W	P	P	N	P	N	N	N
Sample 23	N	P	N	P	P	P	N	P	P	N	N
Sample 29	P	P	N	N	P	P	N	N	N	P	N
Sample 56	P	P	P	N	P	P	N	N	N	P	N
Sample 58	N	P	P	N	P	P	N	N	N	P	N
Sample 85**	N	P	P	P	P	P	N	N	N	N	N
Sample 87*	N	P	N	N	P	P	P	N	N	N	P
Sample 100	N	P	P	N	P	P	N	N	P	N	N
Key: P = Positive band, N = Negative band, W = Weakly reactive band (Not counted as a positive band)											
* *Borrelia burgdorferi* negative, *Borrelia miyamotoi* GlpQ positive											
**C6 ELISA negative, but Western blot positive											