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ORIGINAL RESEARCH

Relapsing fever *Borrelia* in California: a pilot serological study

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Background: *Borrelia* spirochetes are tick-borne Gram-negative bacteria that cause disease in humans and animals. Although many studies have focused on *Borrelia burgdorferi* (Bb), the agent of Lyme disease, recent studies have examined the role of Relapsing Fever *Borrelia* (RFB) in human disease. In this pilot study, we have evaluated serological reactivity against Bb and RFB in patients residing in California.

Methods: Serological testing for reactivity to Bb and RFB antigens was performed in 543 patients with suspected tick-borne illness using a Western blot technique. Further evaluation of a subset of 321 patients residing in California was obtained. Serum samples were tested for IgM and IgG antibodies reactive with Bb and RFB, and samples were classified by county of residence according to Bb reactivity alone, RFB reactivity alone, and dual reactivity against Bb and RFB. Seroreactivity was ranked in counties with the highest absolute number and the highest prevalence of positive samples.

Results: Of the 543 total serum samples, 32% were positive for Bb, 22% were positive for RFB, and 7% were positive for both Bb and RFB. Of the 321 serum samples from patients residing in California, 33% were positive for Bb, 27% were positive for RFB, and 11% were positive for both Bb and RFB. In the California cohort, the highest rates of positive serological testing for Bb were found in Santa Clara, Alameda, and Contra Costa counties, while the highest rates of positive serological testing for RFB were found in Santa Clara, Alameda, Marin, and San Francisco counties. The highest rates of dual reactivity against Bb and RFB were found in Contra Costa, Alameda, and San Francisco counties. Among the 24 counties with patients who were tested, Bb seropositivity alone was found in four counties, RFB seropositivity alone was found in two counties, and seropositivity for both Bb and RFB was found in 14 counties.

Conclusion: Results of this pilot study suggest that seroreactivity against Bb and RFB is widespread in California, and dual exposure to Bb and RFB may complicate the diagnosis of tick-borne disease. Greater awareness of RFB and broader screening for this tick-borne infection is warranted.

Keywords: Lyme disease, *Borrelia burgdorferi*, relapsing fever *Borrelia*, *Borrelia miyamotoi*, tick-borne disease

Video abstract

Point your SmartPhone at the code above. If you have a QR code reader the video abstract will appear. Or use: http://youtu.be/CQKpmB2bhYs

Introduction

Borrelia spirochetes are best known for causing Lyme disease (LD), a tick-borne infection acquired from the bite of an *Ixodes* tick. The *Borrelia* spirochete complex encompasses approximately 52 species of *Borrelia*, of which 21 fall into the LD group (*Borrelia burgdorferi*, Bb) and 29 fall into the Relapsing Fever *Borrelia* (RFB) group that includes the agents of tick-borne and louse-borne relapsing fever.[1–3] Two species remain unclassified.[4]

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In the USA, several species of RFB have been reported to cause disease in humans, including *B. miyamotoi, B. hermsii*, *B. lonestari*, *B. parkeri*, and *B. turicatae*, with most cases occurring in the western USA.[1,2,5–9] In the state of California, *B. miyamotoi*, *B. hermsii,* and *B. parkeri* have been shown to infect humans, and a fourth *Borrelia* species, *B. coriaceae*, infects ticks found in that state, although human infection has not yet been identified.[10] Many of these RFB species are difficult to culture, and this limitation has hindered research into the diagnosis and treatment of infection with these spirochetes.[1] Due to the emergence of genetically diverse RFB, infected individuals often present with a spectrum of symptoms, making diagnosis a challenge for clinicians unfamiliar with the disease.

Starting in 2016, our practice has performed serological testing for RFB on patients with suspected tick-borne disease. Our findings indicate that the genotypic makeup of spirochetal infection in the USA may be more complex than acknowledged at present.

Materials and methods

Patients and data collection

Between October 2016 and May 2018, patients were recruited from a medical practice located in San Francisco, CA, specializing in the diagnosis and treatment of tick-borne diseases. Patients of either sex qualified for the study if they were at least 18 years old and reported musculoskeletal, neuropsychiatric and/or cardiac symptoms consistent with LD.[11] Since the primary goal was to assess exposure and not necessarily active infection with select *Borrelia* genospecies, a known tick bite or erythema migrans rash was not required for participation in the study. Written informed consent for data collection was obtained from each patient, and the anonymous retrospective data collection protocol was approved by the Western Institutional Review Board (WIRB), Puyallup, WA. Blood was drawn at independent laboratories including BioReference® (Elmwood Park, NJ, USA), Laboratory Corporation of America® (Burlington, NC, USA), and AnyLabTestNow® (Alpharetta, GA, USA), and serum samples were sent via overnight mail for tick-borne disease testing. Anonymous patient samples from California were coded according to the patient's county of residence.

Laboratory assessment

Testing for Bb and RFB was performed through IGeneX Clinical Laboratory in Palo Alto, CA. IGeneX is a high-complexity testing laboratory that has Clinical Laboratory Improvement Amendments (CLIA) certification. Serological testing for Bb and RFB was performed using Western blot techniques that detect IgM and IgG antibodies.[12,13] The Bb Western blot detects antibodies reactive with two strains of *B. burgdorferi* (B31 and 297), as previously described.[12] The RFB Western blot detects antibodies reactive with two species of relapsing fever spirochetes, *B. hermsii* and a fast-growing strain related to *B. turcica*, that are representative of RFB species known to infect humans.[13] Since many RFB species are difficult to culture, the two RFB variants were chosen because they could be grown in the laboratory to serve as reliable test substrates. The reports for RFB Western blot testing in this study did not distinguish between the species.

A positive IgM or IgG test for Bb was based on seroreactivity with six significant protein bands on the Western blot, as previously described.[12] The significant bands have molecular weights of 23–25, 31, 34, 39, 41, and 83–93 kDa. The Western blot was interpreted as positive if at least two of the six bands were detected, but with the following exceptions: the test was interpreted as indeterminate if only bands 31 and 41 kDa or only bands 31 and 83–93 kDa were detected. The test was interpreted as negative if only bands 41 and 83–93 kDa were detected, or if less than two bands were detected.

A positive IgM or IgG test for RFB was based on seroreactivity with protein bands from either *B. hermsii* or the *B. turcica*-related strain on the Western blot. An aliquot equivalent to 36 µg of sonicated spirochete cell lysate (300 µg/mL) was fractionated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) on 11.3% acrylamide gel and transferred to Protran BA nitrocellulose membrane (Schleicher & Schuell, Keene, NH) in Tris-glycine-methanol transfer buffer pH 8.3 [25 mM Tris, 192 mM glycine, 20% (vol/vol) methanol]. Detection of serum antibodies against the fractionated RFB protein bands was performed as described for Bb.[12] RFB IgM and IgG Western blots were considered positive if test sera reacted with two of the following four antigens from *B. hermsii*: 21–23, 37 (GlpQ), 41, and 70–75 kDa. RFB IgM and IgG Western blots were considered positive if test sera reacted with two of the following four antigens from the *B. turcica*-related strain: 21–23, 41, 45 (GlpQ), and 70–75 kDa. The test was interpreted as negative if less than two protein bands were detected. Interpretation was based on internal validation studies using confirmed positive and negative serum samples.

Results

A summary of Bb and RFB testing for all patients is shown in Table 1. Serum samples from a total of 543 patients were collected from October 2016 through May 2018 and tested for IgM and IgG antibodies against Bb and RFB. Of these patients, 511 resided in the USA, 22 were residents

of Mexico, six resided in Canada, two were residents of Ireland, one resided in Denmark and one was a resident of New Zealand. Serological testing for Bb yielded 171/543 (32%) positive results, 94/543 (17%) indeterminate results and 278/543 (51%) negative results. Among the samples tested, 54 (10%) had an isolated IgM response, 98 (18%) had an isolated IgG response, and 19 (3%) had a combined IgM/IgG response. Serological testing for RFB yielded 121/543 (22%) positive results and 422/543 (78%) negative results. Among the samples tested, 61 (11%) had an isolated IgM response, 53 (10%) had an isolated IgG response, and 7 (1%) had a combined IgM/IgG response. Positive testing for both Bb and RFB was found in 39/543 (7%) of the serum samples (Table 1).

A summary of Bb and RFB testing for patients residing in California is shown in Table 2. Patients residing in California comprised 321/543 (59%) of the total tested. Serological testing for Bb yielded 106/321 (33%) positive results, 67/321 (21%) indeterminate, and 148/321 (46%) negative results. Among the samples tested, 37 (12%) had an isolated IgM response, 63 (19%) had an isolated IgG response, and 6 (2%) had a combined IgM/IgG response. Serological testing for RFB yielded 87/321 (27%) positive results and 234/321 (73%) negative results. Among the samples tested, 52 (17%) had an isolated IgM response, 29 (9%) had an isolated IgG response, and 6 (2%) had a combined IgM/IgG response. Positive testing for both Bb and RFB was found in 36/321 (11%) of the serum samples (Table 2).

The results of Bb Western blot reactivity, sorted by California counties into positive IgM, IgG, and combined IgM/IgG responses and total positive, indeterminate, and negative responses are summarized in Table 3. Positive test results for Bb seroreactivity were obtained for patients residing in 18 California counties. The counties with the highest numbers of Bb responses were: Santa Clara, 19 positive results; San Francisco, 16 positive results; Alameda, 15 positive results; and Contra Costa, 14 positive results.

The results of RFB Western blot reactivity, sorted by California counties into positive IgM, IgG and combined IgM/IgG responses, and total positive and negative responses are summarized in Table 4. Positive test results for RFB seroreactivity were obtained for patients residing in 16 California counties. The counties with the highest numbers of RFB responses were: Santa Clara, 21 positive results; Alameda, 16 positive results; Marin, 12 positive results; and San Francisco, 12 positive results.

The results of individual Bb, RFB, and dual Bb and RFB seroreactivity sorted by California county are summarized in Table 5. Positive test results for dual Bb and RFB seroreactivity were obtained for patients residing in 11 California counties. Counties with the highest numbers of patients with dual positive Bb and RFB results were: Contra Costa, eight positive results; Alameda, seven positive results; San Francisco, seven positive results; and Santa Clara, six positive results.

The prevalence of Bb, RFB, and dual Bb and RFB seroreactivity in California counties is shown in Table 6. The population estimates for these counties were derived from financial records as of January 1, 2018 (http://www.dof.ca.gov/Forecasting/Demographics/Estimates/E-1/). For the 24 counties tested, the highest overall prevalence of *Borrelia* seroreactivity was found in Marin, Humboldt, and Santa Cruz counties. The highest prevalence of Bb seroreactivity was found in Mendocino, Napa, and Santa Cruz counties, while the highest prevalence of RFB seroreactivity was found in Marin, Humboldt, and Santa Clara counties. The reason for the discrepancy between Bb and RFB county prevalence is unclear at present.

Table 1 Total Lyme and RFB Western Blot Summary, October 2016 through May 2018

Western blot	IgM	IgG	IgM/G	Indeterminate	Negative	Total positive[a]
Lyme	54 (10%)	98 (18%)	19 (3%)	94 (17%)	278 (51%)	171 (32%)
RFB	61 (11%)	53 (10%)	7 (1%)	0	422 (78%)	121 (22%)

Notes: [a]Combined Lyme/RFB in 39 positive cases (7% of total).
Abbreviation: RFB, Relapsing Fever *Borrelia*.

Table 2 California Lyme and RFB Western Blot Summary, October 2016 through May 2018

Western blot	IgM	IgG	IgM/G	Indeterminate	Negative	Total positive[a]
Lyme	37 (12%)	63 (19%)	6 (2%)	67 (21%)	148 (46%)	106 (33%)
RFB	52 (17%)	29 (9%)	6 (2%)	0	234 (73%)	87 (27%)

Notes: [a]Combined Lyme/RFB in 36 positive cases (11% of total).
Abbreviation: RFB, Relapsing Fever *Borrelia*.

Table 3 Lyme Western Blot by California County, October 2016 through May 2018

County	n	IgM	IgG	IgM/G	Indeterminate	Negative	Total positive
Alameda	27	4 (15%)	8 (30%)	3 (11%)	6 (22%)	6 (22%)	15 (56%)
Contra Costa	25	2 (8%)	11 (44%)	1 (4%)	1 (4%)	10 (40%)	14 (56%)
El Dorado	4	2 (50%)	0 (0%)	0 (0%)	0 (0%)	2 (50%)	2 (50%)
Fresno	17	0 (0%)	5 (29%)	0 (0%)	8 (47%)	4 (24%)	5 (29%)
Humboldt	11	0 (0%)	1 (9%)	0 (0%)	2 (18%)	8 (73%)	1 (9%)
Imperial	2	0 (0%)	0 (0%)	0 (0%)	0 (0%)	2 (100%)	0 (0%)
Los Angeles	14	4 (29%)	4 (29%)	1 (7%)	2 (14%)	3 (21%)	9 (64%)
Marin	28	2 (7%)	1 (4%)	0 (0%)	4 (14%)	21 (75%)	3 (11%)
Mendocino	2	2 (100%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	2 (100%)
Napa	3	0 (0%)	2 (67%)	0 (0%)	1 (33%)	0 (0%)	2 (67%)
Nevada	2	0 (0%)	1 (50%)	0 (0%)	1 (50%)	0 (0%)	1 (50%)
Orange	9	2 (22%)	0 (0%)	0 (0%)	0 (0%)	7 (78%)	2 (22%)
Placer	5	1 (20%)	0 (0%)	0 (0%)	2 (40%)	2 (40%)	1 (20%)
Sacramento	5	0 (0%)	0 (0%)	0 (0%)	0 (0%)	5 (100%)	0 (0%)
San Diego	2	0 (0%)	0 (0%)	0 (0%)	2 (100%)	0 (0%)	0 (0%)
San Francisco	48	6 (13%)	9 (19%)	1 (2%)	9 (19%)	23 (48%)	16 (33%)
San Joaquin	5	0 (0%)	1 (20%)	0 (0%)	4 (80%)	0 (0%)	1 (20%)
San Mateo	12	3 (25%)	0 (0%)	0 (0%)	4 (33%)	5 (42%)	3 (25%)
Santa Clara	66	4 (6%)	15 (23%)	0 (0%)	15 (23%)	32 (48%)	19 (29%)
Santa Cruz	19	4 (21%)	2 (11%)	0 (0%)	4 (21%)	9 (47%)	6 (32%)
Solano	4	0 (0%)	0 (0%)	0 (0%)	2 (50%)	2 (50%)	0 (0%)
Sonoma	8	1 (13%)	3 (38%)	0 (0%)	0 (0%)	4 (50%)	4 (50%)
Sutter	1	0 (0%)	0 (0%)	0 (0%)	0 (0%)	1 (100%)	0 (0%)
Tehama	2	0 (0%)	0 (0%)	0 (0%)	0 (0%)	2 (100%)	0 (0%)
Total	321	37 (12%)	63 (20%)	6 (2%)	67 (21%)	148 (46%)	106 (33%)

Table 4 RFB Western Blot by California County, October 2016 through May 2018

County	n	IgM	IgG	IgM/G	Indeterminate	Negative	Total positive
Alameda	27	13 (48%)	3 (11%)	0 (0%)	0 (0%)	11 (41%)	16 (59%)
Contra Costa	25	4 (16%)	1 (4%)	3 (12%)	0 (0%)	17 (68%)	8 (32%)
El Dorado	4	0 (0%)	0 (0%)	1 (25%)	0 (0%)	3 (75%)	1 (25%)
Fresno	17	0 (0%)	2 (12%)	0 (0%)	0 (0%)	15 (88%)	2 (12%)
Humboldt	11	4 (36%)	0 (0%)	0 (0%)	0 (0%)	7 (64%)	4 (36%)
Imperial	2	0 (0%)	1 (50%)	0 (0%)	0 (0%)	1 (50%)	1 (50%)
Los Angeles	14	1 (7%)	0 (0%)	0 (0%)	0 (0%)	13 (93%)	1 (7%)
Marin	28	8 (29%)	4 (14%)	0 (0%)	0 (0%)	16 (57%)	12 (43%)
Mendocino	2	0 (0%)	0 (0%)	0 (0%)	0 (0%)	2 (100%)	0 (0%)
Napa	3	0 (0%)	0 (0%)	0 (0%)	0 (0%)	3 (100%)	0 (0%)
Nevada	2	1 (50%)	0 (0%)	0 (0%)	0 (0%)	1 (50%)	1 (50%)
Orange	9	0 (0%)	0 (0%)	0 (0%)	0 (0%)	9 (100%)	0 (0%)
Placer	5	2 (40%)	0 (0%)	0 (0%)	0 (0%)	3 (60%)	2 (40%)
Sacramento	5	0 (0%)	0 (0%)	0 (0%)	0 (0%)	5 (100%)	0 (0%)
San Diego	2	0 (0%)	0 (0%)	0 (0%)	0 (0%)	2 (100%)	0 (0%)
San Francisco	48	5 (10%)	5 (10%)	2 (4%)	0 (0%)	36 (75%)	12 (25%)
San Joaquin	5	0 (0%)	0 (0%)	0 (0%)	0 (0%)	5 (100%)	0 (0%)
San Mateo	12	1 (8%)	0 (0%)	0 (0%)	0 (0%)	11 (92%)	1 (8%)
Santa Clara	66	10 (15%)	11 (17%)	0 (0%)	0 (0%)	45 (68%)	21 (32%)
Santa Cruz	19	0 (0%)	2 (11%)	0 (0%)	0 (0%)	17 (89%)	2 (11%)
Solano	4	0 (0%)	0 (0%)	0 (0%)	0 (0%)	4 (100%)	0 (0%)
Sonoma	8	2 (25%)	0 (0%)	0 (0%)	0 (0%)	6 (75%)	2 (25%)
Sutter	1	1 (100%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	1 (100%)
Tehama	2	0 (0%)	0 (0%)	0 (0%)	0 (0%)	2 (100%)	0 (0%)
Total	321	52 (16%)	29 (9%)	6 (2%)	0 (0%)	234 (73%)	87 (27%)

Abbreviation: RFB, Relapsing Fever *Borrelia*.

Table 5 Lyme, RFB, and Lyme/RFB Western Blot Summary by California County, October 2016 through May 2018

County	n	Lyme positive	RFB positive	Lyme + RFB
Alameda	27	8 (30%)	9 (33%)	7 (26%)
Contra Costa	25	6 (24%)	0 (0%)	8 (32%)
El Dorado	4	1 (25%)	0 (0%)	1 (25%)
Fresno	17	3 (18%)	0 (0%)	2 (12%)
Humboldt	11	1 (9%)	4 (36%)	0 (0%)
Imperial	2	0 (0%)	1 (50%)	0 (0%)
Los Angeles	14	8 (57%)	0 (0%)	1 (7%)
Marin	28	3 (11%)	12 (43%)	0 (0%)
Mendocino	2	2 (100%)	0 (0%)	0 (0%)
Napa	3	2 (67%)	0 (0%)	0 (0%)
Nevada	2	0 (0%)	0 (0%)	1 (50%)
Orange	9	2 (22%)	0 (0%)	0 (0%)
Placer	5	0 (0%)	1 (20%)	1 (20%)
Sacramento	5	0 (0%)	0 (0%)	0 (0%)
San Diego	2	0 (0%)	0 (0%)	0 (0%)
San Francisco	48	9 (19%)	5 (10%)	7 (15%)
San Joaquin	5	1 (20%)	0 (0%)	0 (0%)
San Mateo	12	3 (25%)	1 (8%)	0 (0%)
Santa Clara	66	13 (20%)	15 (23%)	6 (9%)
Santa Cruz	19	5 (26%)	1 (5%)	1 (5%)
Solano	4	0 (0%)	0 (0%)	0 (0%)
Sonoma	8	3 (38%)	1 (13%)	1 (13%)
Sutter	1	0 (0%)	1 (100%)	0 (0%)
Tehama	2	0 (0%)	0 (0%)	0 (0%)
Total	321	70 (22%)	51 (16%)	36 (11%)

Abbreviation: RFB, Relapsing Fever *Borrelia*.

Table 6 Prevalence of Positive Lyme, RFB, and Lyme/RFB Western Blots by California County, October 2016 through May 2018

County and number tested	Population	Total positive (per 100,000)	Lyme positive (per 100,000)	RFB positive (per 100,000)	Lyme + RFB (per 100,000)
Alameda, 27	1,660,202	24 (1.45)	8 (0.48)	9 (0.54)	7 (0.42)
Contra Costa, 25	1,149,363	14 (1.22)	6 (0.52)	0 (0)	8 (0.70)
El Dorado, 4	188,399	2 (1.06)	1 (0.53)	0 (0)	1 (0.53)
Fresno, 17	1,007,229	5 (0.50)	3 (0.30)	0 (0)	2 (0.20)
Humboldt, 11	136,002	5 (**3.68**)	1 (0.74)	4 (**2.94**)	0 (0)
Imperial, 2	190,624	1 (0.52)	0 (0)	1 (0.52)	0 (0)
Los Angeles, 14	10,283,729	9 (0.09)	8 (0.08)	0 (0)	1 (0.01)
Marin, 28	263,886	15 (**5.68**)	3 (1.14)	12 (**4.55**)	0 (0)
Mendocino, 2	89,299	2 (2.24)	2 (**2.24**)	0 (0)	0 (0)
Napa, 3	141,294	2 (1.42)	2 (**1.42**)	0 (0)	0 (0)
Nevada, 2	99,155	1 (1.01)	0 (0)	0 (0)	1 (1.01)
Orange, 9	3,221,103	2 (0.06)	2 (0.06)	0 (0)	0 (0)
Placer, 5	389,532	2 (0.51)	0 (0)	1 (0.26)	1 (0.26)
Sacramento, 5	1,529,501	0 (0)	0 (0)	0 (0)	0 (0)
San Diego, 2	3,337,456	0 (0)	0 (0)	0 (0)	0 (0)
San Francisco, 48	883,963	21(2.38)	9 (1.02)	5 (0.57)	7 (0.79)
San Joaquin, 5	758,744	1 (0.13)	1 (0.13)	0 (0)	0 (0)
San Mateo, 12	774,155	4 (0.52)	3 (0.39)	1 (0.13)	0 (0)
Santa Clara, 66	1,956,598	34 (1.74)	13 (0.66)	15 (**0.77**)	6 (0.31)
Santa Cruz, 19	276,864	7 (**2.53**)	5 (**1.81**)	1 (0.36)	1 (0.36)
Solano, 4	439,793	0 (0)	0 (0)	0 (0)	0 (0)
Sonoma, 8	503,332	5 (0.99)	3 (0.60)	1 (0.20)	1 (0.20)
Sutter, 1	97,238	1 (1.03)	0 (0)	1 (1.03)	0 (0)
Tehama, 2	64,039	0 (0)	0 (0)	0 (0)	0 (0)
Total 321	29,441,410	157 (0.53)	70 (0.24)	51 (0.17)	36 (0.12)

Notes: County population estimates were derived from financial records (http://www.dof.ca.gov/Forecasting/Demographics/Estimates/E-1/). Numbers in bold represent counties with the highest prevalence of Lyme and/or RFB.
Abbreviation: RFB, Relapsing Fever *Borrelia*.

Figure 1 shows a Venn diagram of the 321 California patients who were tested for Bb and RFB in the study. Of the 157 patients who were seropositive for Bb and/or RFB, 70 (45%) were seropositive for Bb alone, 51 (32%) were seropositive for RFB alone, and 36 (23%) were seropositive for both Bb and RFB. There were 164 patients who were seronegative for both Bb and RFB.

Figure 2 shows the distribution of California counties with patients who were positive for Bb alone, RFB alone, and both Bb and RFB. Patients from 24 counties were tested. Bb testing alone was positive in patients from four counties, RFB testing alone was positive in patients from two counties, Bb and RFB testing was positive in patients from 14 counties, and no positive testing was found in patients from four counties.

Discussion

Studies of humans and reservoir hosts in California suggest that RFB may be a growing problem in the state. Krause et al conducted a retrospective study of sera collected between 1988 and 1989 from residents of southern Mendocino County at high risk for LD. Their study showed that RFB was present in 2%–7% of those individuals decades before infection with RFB was even recognized. Infection with *B. miyamotoi* and *B. hermsii* could not be distinguished with certainty, but findings suggested that both RFB species were present in that county.[10] Two studies of rodents in Southern California using serology and molecular detection methods in 2009 and 2014 found that up to 7.7% were infected with RFB.[7,14] *B. miyamotoi* was also found in *Ixodes pacificus* ticks from 24 of 48 California counties that were surveyed over a 13 year period, and the prevalence of this RFB strain in adult ticks was similar to the prevalence of Bb.[15]

Our serological study of California patients who were tested for tick-borne disease suggests that Bb and RFB may have a similar prevalence in these patients (33%vs 27%), and evidence for dual seropositivity with Bb and RFB was found in 11% of patients (Table 2 and Figure 1). Seropositivity for Bb was noted in patients from 18 of 24 counties surveyed, while seropositivity for RFB was found in 16 of 24 counties surveyed. Patients who were seropositive for Bb and RFB were found in 14 counties, while dual seropositivity for both Bb and RFB was noted in patients from 11 counties (Tables 3–5). Although our numbers are small so far, the results suggest that RFB could be widespread in California based on the limited data available at present (Table 6 and Figure 2).

A possible concern is serological cross-reactivity between Bb and RFB antigens on Western blots. Cross-reactivity is unlikely for the following reasons: First, when we tested Bb-reactive rabbit serum on the RFB Western blot and RFB-reactive rabbit serum on the Bb Western blot, we found that only the 41 kDa antigen was weakly cross-reactive. The other five significant Bb proteins and the other three significant RFB proteins did not cross-react (data not shown). Second, we found that 89% of patients in California had seroreactivity to either Bb or RFB antigens but not both, and the lack of widespread dual reactivity indicates that cross-reactivity in our distinct Western blot format was unlikely.

B. miyamotoi, a relapsing fever spirochete that was first identified in Japan over 20 years ago, was thought to have been recently introduced into the Western hemisphere.[9,15]

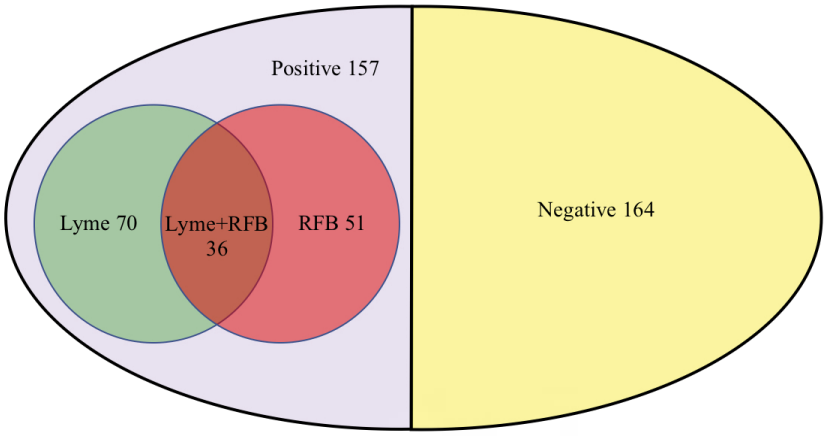

Figure 1 Venn diagram of 321 California patients tested for Lyme, RFB, and dual Lyme + RFB seropositivity, October 2016 through May 2018.
Abbreviation: RFB, Relapsing Fever *Borrelia*.

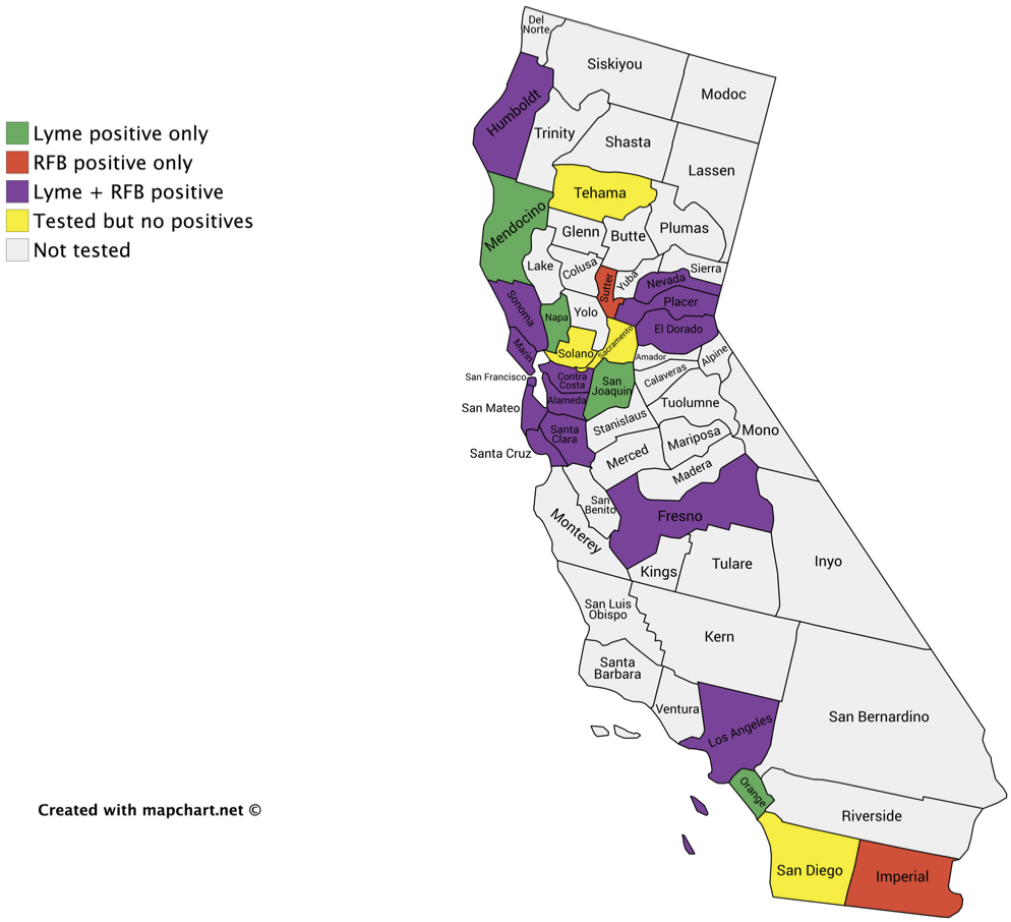

Figure 2 Map showing distribution of Lyme, RFB and Lyme + RFB testing in California counties, October 2016 through May 2018.
Notes: Patients from 24 counties were tested. Lyme testing alone was positive in four counties, RFB testing alone was positive in two counties, Lyme + RFB testing was positive in 14 counties, and no positive testing was found in four counties. Map created using https://mapchart.net/.[45]
Abbreviation: RFB, Relapsing Fever *Borrelia*.

B. miyamotoi infection has a prevalence of 1%–3% in the northeastern USA, but until recently had never been thought to be on the West Coast. With advances in molecular testing, however, identification of specific *Borrelia* spp. now challenges pre-existing dogma surrounding the geographic distribution and occurrence of RFB.[16] A recent study from Russia found that about 23% of *Borrelia* infections were associated with positive molecular testing for *B. miyamotoi*.[17] Between 1987 and 2000, 450 human cases of RFB, predominantly *B. hermsii*, were reported in the USA, most of which occurred in the western states.[9] In Northwest Morocco, *B. hispanica* was reported to have caused over 20% of unexplained fever cases, and in Senegal *B. crocidurae* infection had a reported incidence of 14 per 100 person-years.[9]

Interestingly, *B. miyamotoi* and *B. lonestari* are transmitted by hard ticks, leading to the proposed distinction of hard tick-borne relapsing fever (HTBRF) or *B. miyamotoi* disease (BMD).[1,10,18,19] In the USA, *B. miyamotoi* strains have been detected in the hard ticks *Ixodes scapularis* and *Ixodes pacificus*,[6,9,20,21] while *B. lonestari* DNA has been detected in the hard tick *Amblyomma americanum*.[5] *B. miyamotoi* also varies genetically depending on geographic region, leading to the concept of the *B. miyamotoi* sensu lato complex.[6,19,22–26]

The genetic diversity of *Borrelia* spp. has consequences for the performance of LD diagnostic tests, and this is reflected in the dismal sensitivity of commercial serological testing.[27,28] To date, most commercially available kits for serological testing are based on detection of just one Bb strain, B31.[12,28–31] Testing for *Borrelia* spirochetes ideally should include the entire spectrum of organisms that encompass both Bb and RFB. RFB infection is only sporadically detected because commercially available serologic testing is not readily available.[32] The development of testing that reflects the complexity and diversity of *Borrelia* species worldwide is

essential for improving the accuracy of LD diagnosis and treatment (Table 1).

RFB should be considered a major public health concern as infected individuals can develop cyclical fevers with flu-like symptoms and possible central nervous system involvement, especially in immunocompromised patients.[19] Dissemination of the RFB spirochete within the bloodstream occurs at a rate 100–1000 times that of the Lyme spirochete and results in a mortality rate of approximately 4%–10% if left untreated, particularly in species endemic to Asia and Africa.[33,34] While blood smears obtained at times when the patient is febrile may be diagnostic, blood microscopy can be complicated by the presence of pseudospirochetes, which are filaments derived from erythrocytes that can be confused with living spirochetes such as *Leptospira* and *Borrelia*.[35,36] Thus, diagnosis through microscopy should ideally be confirmed by other more specific testing such as serological assays.

As with Bb, infection with RFB requires prompt antibiotic treatment to ensure a positive clinical response, and antibiotic therapy can trigger a Jarisch–Herxheimer reaction.[37,38] Symptoms of *Borrelia* and other tick-borne infections are not specific, and patients can have mixed infections with significant overlap of symptoms.[39,40] Testing for both Bb and RFB allows a greater diversity of *Borrelia* genotypes to be detected and will enhance our understanding of geographical distribution and infection rates through surveillance and monitoring.

Our study found that Bb-seropositive patients tended to have more frequent IgG reactivity, while RFB-seropositive patients tended to have more frequent IgM reactivity (Table 2). Although this difference may reflect the timing of testing, persistent IgM reactivity has been reported in patients with LD and suggests that Bb is viable throughout the course of the illness.[41–43] The patients in this study were evaluated for Bb and RFB exposure because they had symptoms consistent with tick-borne infection, such as musculoskeletal, neuropsychiatric and/or cardiac problems. Persistent Bb infection despite treatment with antibiotics coupled with prolonged IgM reactivity has been found in humans.[42,44] Our study suggests that prolonged IgM reactivity may be a feature of RFB infection as well and suggests that this infection may be persistent. Recognition of prolonged IgM reactivity to RFB should shape recommended testing protocols for accurate diagnosis.

Our study has a number of limitations. RFB seropositivity does not necessarily indicate active infection, and conversely some RFB-infected patients may be seronegative. The presence of positive serology indicates exposure to the spirochete, however, and testing for active infection using other methods should be considered based on the serological results. The number of subjects in our study is small and this limitation is particularly relevant when applying figures by county. The county of residence does not necessarily indicate that the patient was infected in that county, but the demographics provide a basic idea of where RFB-exposed patients can be found. In addition, the Western blot used in this study does not indicate the exact RFB species detected in each case, and reactivity with other RFB species might have been missed. Thus, our pilot study may have underestimated the prevalence of RFB exposure in our patients. More sophisticated immunoblot testing will determine the exact RFB species and will help to refine our understanding of RFB prevalence in California.

In summary, exposure to Bb and RFB appears to be a growing concern in California. RFB infection may explain "Lyme-like" symptoms in patients who are seronegative for Bb, and dual infection with Bb and RFB may confound the diagnosis of tick-borne disease. Greater awareness of RFB and broader screening for this tick-borne infection is warranted.

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Author contributions

All authors contributed toward data analysis, drafting, and revising the paper and agree to be accountable for all aspects of the work.

Disclosure

JSS is President and Laboratory Director of IGeneX Clinical Laboratory, Palo Alto, CA. RBS is the owner of Union Square Medical Associates, a medical practice that treats tick-borne diseases in San Francisco, CA. The other authors report no conflicts of interest in this work.

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