Disparity Between Serological Reactivity to *Borrelia burgdorferi* and Evidence of Past Disease in a High-Risk Group

Fernando Arteaga, Marc G. Golightly, Ana García Perez, Marta Barral, Pedro Anda, and Juan C. García-Monco

From Servicio de Neurología, Hospital de Galdacano, Vizcaya, and Instituto de Salud Carlos III, Majadahonda, Madrid, Spain; the Department of Pathology, State University of New York at Stony Brook, Stony Brook, New York, USA; and SIMA, Derio, Vizcaya, Spain

A prevalence study of past Lyme borreliosis in persons with outdoor occupations was done. Consenting individuals (*n* = 302) were administered a questionnaire eliciting demographic and occupational data and a clinical history, and were asked to donate a serum specimen for detection of antibodies to *Borrelia burgdorferi* by enzyme-linked immunosorbent assay (ELISA), immunoblotting, and borrelia inhibition assays, and for detection of potentially cross-reactive antibodies. Of 302 individuals, 77 (25%) had reactive antibodies detected by ELISA. Of these 302 individuals, 44 (15%) met the criteria of the Centers for Disease Control and Prevention for serological reactivity as evidenced by immunoblotting, and 70 (23%) had inhibitory activity. Through the clinical criteria employed, only 11 individuals with serological reactivity had prior illness compatible with Lyme borreliosis. Higher ELISA absorbances were positively correlated with age and duration of outdoor occupation. The results from three serological assays and the lack of reactivity to potentially cross-reactive infectious agents indicate that serological reactivity was due to exposure to *B. burgdorferi*. The disparity between serological reactivity and the clinical evidence of Lyme borreliosis suggests cumulative exposure to a nonpathogenic form of *B. burgdorferi*.

Lyme borreliosis is a tick-borne multisystemic disorder that can affect the skin, heart, and the nervous and musculoskeletal systems [1–3]. It has long been known that outdoor occupations are associated with a high risk for this infection because of greater contact with the vector ticks. Prior serological surveys in Europe have shown a disparity between high levels of serological reactivity and evidence of past disease in persons with outdoor occupations [4–10]. This disparity has been interpreted as an indication of high levels of subclinical illness. Many of the studies that have documented high levels of serological reactivity and a low prevalence of clinical manifestations in high-risk populations were done before the standardization of serological assays. Therefore, these results could also be interpreted as being due to cross-reactions with other common pathogens.

In this study, a risk evaluation was undertaken to document the prevalence of past Lyme borreliosis in a high-risk population, to verify the specificity of serological reactivity to *Borrelia burgdorferi* by using standardized procedures and a standardized case definition [11–13], and to rule out that serological reactivity was due to cross-reactivity with antigenically related or other common bacteria that can be encountered in outdoor occupations in our area. *B. burgdorferi* sensu stricto [14] and *Borrelia garinii* [15] have been isolated from infected *Ixodes ricinus* and from patients with erythema migrans (EM) in northern Spain. The incidence of Lyme disease in our area is not known, but patient series [8, 10] as well as data derived from our own hospital-based practice indicate that Lyme disease in northern Spain could be an important public health problem.

Volunteers and Methods

There were 302 persons enrolled in the study; they had the following outdoor occupations in the province of Vizcaya in northern Spain: forestry workers, 117; large animal veterinarians, 52; shepherds, 18; apiculturists, 27; mushroom and truffle gatherers, 74; and other diverse outdoor activities, 14. The protocols used in this study were approved by the institutional review board for research with human subjects at our hospital. The identity of the volunteers was protected by the substitution of the name with a number in the questionnaire. A signed consent form was obtained from all volunteers before administering the standardized questionnaire and obtaining a blood specimen. The questionnaire sought information on age, sex, demographics, occupation, time spent outdoors as part of their occupation, and duration (years) of outdoor occupation. A comprehensive clinical history emphasizing tick exposure during the last 5 years and the manifestations of Lyme disease and other infections also was obtained.

The volunteers were shown photographs of EM and other skin disorders to assist in the answering of questions on the cutaneous lesion of Lyme borreliosis. Questions were designed to elicit appropriate answers for the various manifestations of
neuroborreliosis [3]. The questionnaire specifically requested information on neurological conditions that usually require medical intervention, including meningitis, encephalitis, Ban- nwarth’s syndrome, and seventh cranial nerve palsy [3]. The questionnaire established a difference between arthralgias and frank arthritis. Specific questions about other diseases (infectious as well as noninfectious) including rheumatoid arthritis, multiple sclerosis, tuberculosis, and syphilis were asked. Reasons for refusing to participate in the study were sought and recorded.

Antibodies to the B31 strain of B. burgdorferi [1] were detected by an alkaline phosphatase–based ELISA and by immunoblotting. For each 96-well plate, the mean absorbance of each serum specimen at a dilution of 1:500 was compared with the mean absorbance of 10 negative controls ±3 SDs (cutoff value). To permit comparisons between plates, the mean absorbance of the serum sample was divided by the cutoff value to obtain a ratio. A ratio of ≥1 represented a reactive specimen, but for a more stringent analysis an absolute absorbance of ≥0.2 was reactive.

Immunoblotting was done with affinity-purified alkaline phosphatase–conjugated antibody to μ chain–specific human IgM and antibody to γ chain–specific human IgG at a serum dilution of 1:100 according to standardized methods and criteria [11, 12]. Specific polypeptides were identified with concurrent immunoblots of murine monoclonal antibodies. In addition, a 24-hour borrelia inhibition assay [16] of the 77 ELISA-positive serum samples and 77 randomly selected ELISA-negative serum samples was done. Rapid plasma reagin tests for syphilis and indirect immunofluorescence for detection of antibodies to Leptospira species were done on all 77 reactive specimens and a random sample of 77 nonreactive serum specimens. The serological reactivity of the 21 most reactive (by ELISA) specimens to Coxiella burnetii and to Brucella abortus, two known pathogens in our rural areas, was determined by CF testing.

Statistical analysis. Pearson correlation coefficients and the χ² test were used for statistical analyses.

Results

There were 302 volunteers recruited for this study, all of whom were adults; 208 (69%) were men, and 94 (31%) were women. The age of the volunteers ranged from 19 to 76 years (19 to 30 years, 18%; 31–40 years, 41%; 41–50 years, 21%; 51–60 years, 10%; 61–70 years, 8%; and 71 or older, 2%).

Serological reactivity to B. burgdorferi by ELISA is shown in table 1. Various degrees of serological reactivity were used. Serum specimens were divided into groups according to the absorbances determined by the initial ELISA. Specimens with a mean absorbance of ≥0.2 (77 serum samples) were most reactive (absorbance range, 0.2 to 1.2). A second group of 29 reactive serum specimens with absorbances of <0.2 but a mean absorbance greater than the cutoff value for the ELISA (ratio, ≥1.0) were considered weakly reactive. Those specimens with mean absorbances lower than the cutoff value were nonreactive.

Ig class–specific immunoblotting was performed on all 106 reactive serum specimens and on an equal number of randomly selected nonreactive serum samples. The criteria of the Centers for Disease Control and Prevention (CDC) for reactivity determined by immunoblotting were used [12]; 44 serum specimens (15% of 302 total samples and 42% of 106 ELISA-reactive samples) had unequivocal profiles in either both the IgM and IgG immunoblots or the IgG immunoblot alone. None of the serum samples nonreactive by ELISA were positive by immunoblotting. In addition, 70 of the 77 ELISA-reactive serum samples tested (table 1) and eight of the 77 randomly selected nonreactive serum samples had an inhibitory effect on the growth of B. burgdorferi in a 24-hour period (χ² test; 1 df; P < .001).

Serological reactivity to B. burgdorferi cannot be explained as a cross-reaction with other infectious agents. Antibodies to Leptospira, Coxiella, and Brucella and reactivity to rapid plasma reagin were not detected in any of the serum specimens tested. Prior tuberculosis was not a possibility according to the questionnaire answers.

The clinical data indicated that a significant number of persons with seroreactive samples had EM and neurological and musculoskeletal manifestations (table 2). For the purposes of analyses, a case of Lyme borreliosis was defined as having had an EM and one systemic manifestation (neurological, musculoskeletal, or both). According to this definition, 11 persons with serological reactivity had Lyme borreliosis (table 2), and none of the patients with nonreactivity had Lyme borreliosis, although there were three individuals who gave a history of EM with no additional manifestations. The number of persons with musculoskeletal conditions as a single manifestation was significantly higher in the group with seroreactivity (table 2) than in the group with nonreactivity; this finding could be associated with Lyme borreliosis. Even if all of the persons with musculoskeletal manifestations were included as having true cases, they would account for only 35% of the total number of persons with seroreactivity (table 2). The remaining 65% of these individuals

<table>
<thead>
<tr>
<th>Finding</th>
<th>No. (%) of individuals</th>
</tr>
</thead>
<tbody>
<tr>
<td>ELISA absorbance, ≥0.2</td>
<td>77 (25)</td>
</tr>
<tr>
<td>ELISA absorbance, greater than cutoff value*</td>
<td>29 (10)</td>
</tr>
<tr>
<td>Total with reactivity</td>
<td>106 (35)</td>
</tr>
<tr>
<td>Positive immunoblotting†</td>
<td>44 (15)</td>
</tr>
<tr>
<td>Positive borrelia inhibition assay‡</td>
<td>70 (23)</td>
</tr>
<tr>
<td>Nonreactivity</td>
<td>196 (65)</td>
</tr>
</tbody>
</table>

* Absorbances of <0.2 but greater than cutoff value.
† Seventy-seven serum samples with ELISA absorbances of ≥0.2 and 77 randomly selected nonreactive serum samples were tested.
Thus, on the basis of the results of two separate standardized procedures and one functional assay for detecting antibodies to *B. burgdorferi*, the high level of tick bites, and the lack of cross-reactivity with other infectious agents, we are certain that the seroreactivity in this study was due to exposure to *B. burgdorferi* and cannot be explained as a cross-reaction with other infectious agents. The disparity noted between the high level of serological reactivity and evidence of compatible clinical manifestations (11 individuals met criteria for the case definition [13]) has been reported in other studies in Europe [4–10]. On the basis of these findings, as well as the positive correlation between age and duration of the outdoor occupation, we suggest that the disparity between high levels of serological reactivity and prior evidence of clinical illness may represent a cumulative exposure to nonpathogenic strains of *B. burgdorferi*. The strict case definition that was used in this study could have had the effect of reducing the number of clinical cases and enhanced the disparity with asymptomatic but reactive individuals; likewise, it is clear that, as with any infectious disease, asymptomatic infection is more common than symptomatic disease. On the other hand, the discovery of two non-pathogenic genospecies of *B. burgdorferi sensu lato* in Europe [17, 18] could reinforce the notion that the disparity might also be due to exposure to these or other nonpathogenic *Borrelia* organisms. Given the fact that two pathogenic genospecies of this agent occur in northern Spain [14, 15], a higher rate of clinical manifestations may be expected. Aside from the possibility of widespread pathogenic diversity of this organism, the high prevalence of serological reactivity without disease should be considered by clinicians to avoid overdiagnosis of Lyme borreliosis [19].

**Acknowledgments**

The authors thank Dr. Carmen Saez de Ocariz for performing the serological tests for *Brucella* and *Rickettsia*.

**References**


**Table 2.** Outdoor workers with clinical manifestations compatible with Lyme borreliosis in northern Spain according to serological reactivity.

<table>
<thead>
<tr>
<th>Finding (no. of individuals)</th>
<th>Tick bite (no. (%))</th>
<th>EM (no. (%))</th>
<th>Neurological manifestation (no. (%))</th>
<th>Musculoskeletal manifestation (no. (%))</th>
</tr>
</thead>
<tbody>
<tr>
<td>ELISA absorbance, ñ0.2 (77)</td>
<td>50 (65)</td>
<td>8 (10)</td>
<td>4 (5)</td>
<td>31 (40)</td>
</tr>
<tr>
<td>ELISA absorbance, &lt;ñ0.2 (29)</td>
<td>13 (45)</td>
<td>3 (10)</td>
<td>1 (3)</td>
<td>6 (21)</td>
</tr>
<tr>
<td>Total with reactivity (106)</td>
<td>63 (59)</td>
<td>11 (10)*</td>
<td>5 (5)*</td>
<td>37 (35)*</td>
</tr>
<tr>
<td>Nonreactivity (196)</td>
<td>114 (58)</td>
<td>3 (2)</td>
<td>2 (1)</td>
<td>39 (20)</td>
</tr>
</tbody>
</table>

NOTE. EM = erythema migrans.
* The number of persons with EM was significantly higher in the group with serological reactivity (\( \chi^2 \) test; 1 df; \( P < .001 \)).
† The number of persons with neurological manifestations was significantly higher in the group with serological reactivity (\( \chi^2 \) test; 1 df; \( P < .05 \)).
‡ The number of persons with musculoskeletal manifestations was significantly higher in the group with serological reactivity (\( \chi^2 \) test; 1 df; \( P < .005 \)).

...gave no history of manifestations compatible with Lyme borreliosis despite serological reactivity to *B. burgdorferi* as measured by ELISA, immunoblotting, and borrelia inhibition assays. The number of individuals with tick bites in both the groups with and without seroreactivity was approximately equal.

Pearson correlation coefficients were calculated for age and serological reactivity (ELISA absorbance) for all of the 302 volunteers; a positive correlation (\( r = .179; \ P < .02 \), two-tailed) between increasing age and absorbance was found. The same calculations were made for the groups with and without seroreactivity separately. Higher absorbances were positively correlated with older individuals, thus indicating that the correlation coefficient (\( r = .274 \) had a probability of occurring randomly <0.01% of the time. Likewise, higher absorbances were positively correlated with the duration of the outdoor occupation (\( r = .204; \ P < .02 \)). Apiculturists, shepherds, and forestry workers had the highest number of seroreactive samples, and large-animal veterinarians had the lowest number of seroreactive samples. With the exception of the veterinarians, the persons with other outdoor occupations were unaware of Lyme borreliosis as a clinical entity.

**Discussion**

Of 302 outdoor workers included in this serosurvey, 106 (35%) had reactive antibodies to *B. burgdorferi* as evidenced by ELISA. Immunoblots for 44 (42%) of these 106 individuals met the CDC criteria for serological reactivity [11, 12]. In addition, 70 serum samples inhibited the growth of this organism.