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Brief Communication

Prevalence of antibodies to *Borrelia burgdorferi sensu stricto* in humans from a Cuban village

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Lyme disease has not been officially reported in Cuba. However, clinical cases have been serologically reported. Seroprevalence survey of *Borrelia burgdorferi sensu stricto* antibodies in humans in the country has not been conducted.

Objective: To estimate the prevalence of borrelial antibodies in inhabitants of a village with historically high level of tick infestation.

Methods: Serum specimens from 247 persons randomly selected from the population of the village were examined by IgG Western blot using B31 strain for estimating the prevalence of antibodies profile.

Results: A seroprevalence value interval (95% CI) of 0.6%-7.2% was estimated for the studied population. The prevalent borrelial protein bands on immunoblots were 41, 72, 90/93, 34, 47, 60, 58, 56, 65/66 and 31 kDa in a decreasing order of significance.

Conclusion: These results support the previous serological findings, suggesting the presence of this borreliosis in Cuba.

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Lyme disease, a multisystem inflammatory disorder caused by the spirochaetal complex *Borrelia burgdorferi sensu lato*, is the most frequently reported vector-borne infectious disease in the Northern hemisphere. The human infection is transmitted by ixodid tick bites and caused primarily by three pathogenic genospecies, *B. burgdorferi sensu stricto*, *Borrelia garinii* and *Borrelia afzelii*.¹ In North America, *B. burgdorferi sensu stricto* is the sole cause of the infection; in Europe, the three genospecies can cause the disease, while *B. garinii* and *B. afzelii* can be found in Asia. In addition, other 15 closely related *Borrelia* species have been identified, but only *Borrelia lusitaniae*, *Borrelia valaisiana* and *Borrelia spielmani* cause human infections.¹⁻³

The disorder develops in stages and has different manifestations that mainly involve the skin, nervous systems, heart and joints; although some infected people have no symptoms, up to 20% may not show the characteristic skin manifestations, and many patients also have no (or mild) systemic symptoms at presentation.⁴

The diagnosis of Lyme borreliosis is based on the recognition of typical clinical signs and is assisted by laboratory tests, of which antibody detection methods are preferred.⁵

This illness represents a considerable emerging infectious disease threat because of its consequences to human health and the difficulties in preventing and controlling it.

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The morbidity rates continually increase in some geographical areas. This may be due to the actual spread of the disease and/or alternatively to improved diagnostic methods.⁴

In Cuba, Lyme disease has not been officially reported, although specific antibodies against *B. burgdorferi sensu lato* complex, measured by ELISA and Western blot techniques, have been found in individuals that have presented compatible clinical manifestations with this borreliosis.⁶⁻⁸

The aim of the present study was to estimate the prevalence of specific antibodies to *B. burgdorferi sensu stricto* in a human population from Cuba, in order to continue the investigations about the presence of this pathogen in the country.

The studied population lives in one of the villages located in Sierra del Rosario, in the Eastern part of the mountainous area of Guaniguanico, between Pinar del Rio and Artemisa provinces, Cuba. It is a small rural area with the following climatological conditions: 24.4°C as multiannual average temperature and 2013.9 mm as annual average rainfall.⁹ Such conditions are favorable for the survival of different tick species. This place was selected because it has had high level of tick infestation historically, mainly *Amblyomma cajennense* (a three-host ixodid tick) and because it was there that the first Cuban cases of Lyme disease were serologically reported.^{6,7}

From the total number of inhabitants (n = 980) in this village, 247 were invited to take part in the study from January to May 2006, selected by a random simple sampling. All of them were adults. The serosurvey was conducted with the consent of subjects in compliance with the Declaration of Helsinki. One serum specimen per subject was collected and transported to the ADMed Microbiology Laboratory of La-Chaux-de-Fonds, Switzerland, according to the International Air Transport Association recommendations.

An in-house Western blot, a validated and standardized system used as confirmatory test in the routine diagnosis, was used for the detection of IgG in the sera. Briefly, a bacterial protein suspension was prepared from *B. burgdorferi sensu stricto* (B31) and a protein profile was made by its electrophoretic separation and transferred to nitrocellulose membranes with semi-dry apparatus (Biorad, France), according to standard laboratory procedures. Human sera diluted 1:200 were added to the membranes, followed by the addition of alkaline phosphatase labeled goat anti-human IgG (diluted 1:500) (Kirkegaard & Perry Laboratories, USA). A negative and a positive sample were added for each assay.

The borrelial antibodies profile obtained in the blots for the studied population sample and in the individual blots estimated as positives were analyzed and described.

The North American strain B31 was used as antigen because Cuba is very close, from a geographically viewpoint, to the United States of America, it is in the way of migratory birds and any other genospecies has not been reported in the American continent. It is known that reliability of serological investigations increases when antibodies are measured against the antigens of *Borrelia* spp. occurring in a particular geographic area.¹⁰

Band intensities were determined by visual comparison with band intensities for the positive control, and they were graded in: 0 (when traces were observed), and from 1+ to 4+ according to the intensities. Zero was not counted and given as negative, 1+ was interpreted as weak result and 2+ to 4+ were interpreted as strong results. Data were stored in an Excel sheet (Microsoft Office) for analysis.

According to the ADMed Microbiology laboratory criteria (based on the total addition of the scores assigned to each band, in accordance to the bands' specificities) the seroprevalence rate was 2.02% (5/247) (95% CI, 0.6%-4.7%). The same result was found when we used the Centers for Disease Control and Prevention (CDC) criteria for a Western blot-IgG, criteria recommended in the American region.¹¹ However, when we used the CDC criteria with the inclusion of OspA and OspB proteins suggested by Hilton et al.,¹² the seroprevalence rate was 4.45% (11/247) (95% CI, 1.7%-7.2%). Then, we could estimate a prevalence of specific borrelial antibodies in the studied population of 0.6%-7.2% as result of the union of confidence intervals.

The sera identified as positives were tested by Rapid Plasma Reagin test (Centis Diagnosticos, Cuba), *Treponema pallidum* haemagglutination assay (Centis Diagnosticos, Cuba) and microagglutination test in order to search for cross-reactions with treponemal and leptospiral antibodies, because cross-reactive antibodies to other related (*Leptospira*, *Treponema*, relapsing fever *Borrelia*) and nonrelated (*Salmonella*, *Pseudomonas*, *Neisseria*) bacterial species have been described.¹³ Cross-reactions with leptospiral and treponemal antibodies were not found by the specific tests and relapsing fever is not reported in Cuba.

The presence of specific antibodies in the individuals not always means *Borrelia* disease, due to clinical or subclinical infection occurrence in the past.⁵ Therefore, in our study, people with positive blots suggest previous contact with the infectious agent, since antibodies persist long time after the original infection. The estimated seroprevalence interval in the studied population is relatively low. However, it is necessary to keep in mind that Lyme disease is not officially reported in Cuba; that is why any evidence or finding about it is relevant.

Similar studies in other Latin American countries have been reported. In Mexico, the first study of seroprevalence of IgG antibodies against the strain B31 by Western blot, showed 0.3% as positivity rate during 1999¹⁴ and in a following study, rates of 3.43% and 6.2% were reported in different regions;¹⁵ all these rates are within the confidence interval estimated in our study.

Other non-endemic countries have also reported low rates of seroprevalence in blood donors and higher rates in forestry workers or farmers, providing serological evidences of *B. burgdorferi* infection.^{16,17}

In countries where Lyme borreliosis is frequent, the seroprevalence rates depend on the studied region and on the occupational characteristics of the population. Antibodies against *B. burgdorferi* have been more frequent in agricultural and forestry workers (7.03%) than in blood donors (3.56%). Nevertheless, in forestry rangers from Northeastern Italy the rates are much higher (23.2%).^{18,19} Rates of 31.3% and 12.8% have been reported in populations from a mountainous area of Spain and from Slovakia, respectively.^{20,21} We studied a sample of a population considered at risk, as they inhabit in a rural and mountainous area with a high level of tick infestation during years. A clinical and/or epidemiological correlation with laboratory results was not possible because a questionnaire was not applied to the subjects enrolled in the study, important aspect to consider in future investigations.

The importance of interpreting Western blot results according to the characteristics of Lyme disease in a local area at risk has been emphasized by different authors. Laboratories in countries with a low prevalence of Lyme borreliosis prefer to use

a rule that gives a higher specificity at the expense of sensitivity. Lyme borreliosis is not the same in all geographic areas due to different local prevalence of species and strains of *B. burgdorferi sensu lato* and also to the heterogeneity of strains. For these reasons, the recommendations for the interpretation of blots have not always been applicable to population in geographic areas other than where they were developed.²²

Nineteen antigenic bands, ranged from 14 to 90/93 kDa, were detected on immunoblots, and from 0 to 12 bands were regularly recognized by each serum. Most sera reacted with 4-7 proteins and only a few sera reacted with 8-12 proteins. The average number of bands seen on immunoblots was 5.3 ± 1.9 .

The number of reactive bands on blots was low in comparison with the positive control blots (up to 21 bands were revealed), but was superior than the negative control blots (2-3 faint bands maximum), and this means that some other reactions were developed with different intensity degrees.

Average numbers of reactive bands from 6.7 ± 4.1 to 8.7 ± 4.1 in early Lyme disease patients, 4.1 ± 2.6 in healthy donors and 3.7 ± 2.3

in other illness patients have been reported on IgG immunoblots.²³ The number of reactive proteins in our investigation was superior to those for healthy donors and other ill patients.

Fig. 1 summarizes the frequencies of recognition of each band and its intensities. The 90/93, 72, 60, 56, 47, 41, 34 and 31 kDa bands were significantly revealed on blots, being the bands of 72, 60, 47 and 41 kDa detected in more than 50% of the samples. In most of the blots, the protein bands were recognized at a low intensity interpreting the results as weak.

Fig. 2 shows the results obtained when analyzing only the blots estimated as positive. Ten proteins turned out as significant for the IgG immunoblot. In decreasing order of significance, these were 41, 72, 90/93, 34, 47, 60, 58, 56, 65/66 and 31 kDa. The 41, 90 and 72 kDa proteins were detected in all positive tested sera. The estimated positive sera gave reactions with 8 or more protein bands and the stronger results were more frequent, although the reactivity of specific bands for *B. burgdorferi sensu stricto* was superior in the zones of 90/93 kDa and 58-56 kDa.

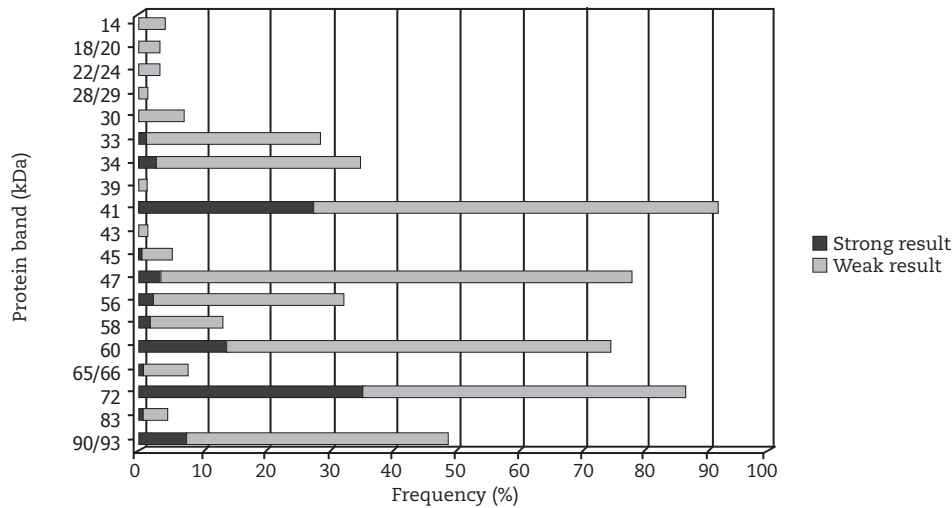


Fig. 1 – Frequencies of recognition and intensity of the borrelial protein bands on immunoblots (n = 247) using sera from the studied population.

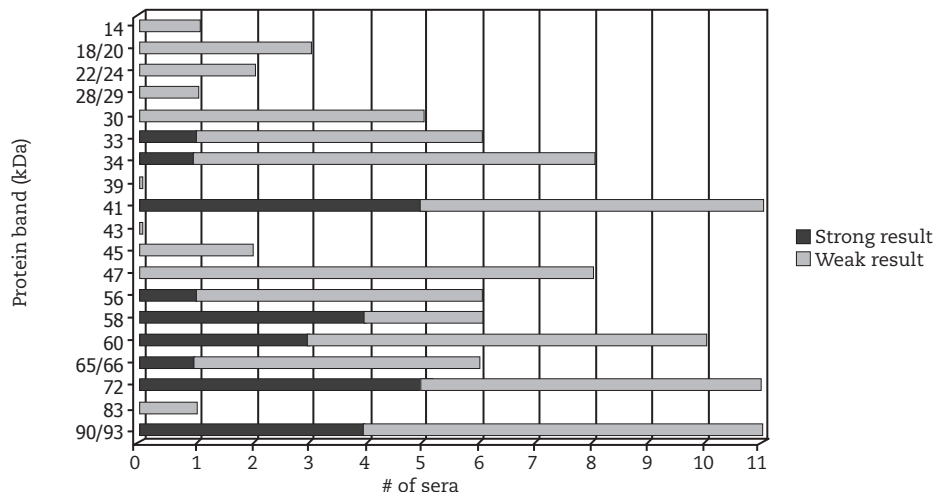


Fig. 2 – Recognition and intensity of the borrelial protein bands by the sera and estimated as positives on immunoblots (n = 11).

Antibodies to highly specific and non-specific borrelial proteins were detected, but the analysis of these should not be done in an individual way because there are non-specific proteins that have value when they are detected together with specific proteins. Thus, the 72 kDa heat shock protein and 41 kDa flaggellin-proteins, the mainly revealed proteins in this study, are not specific for *Borrelia* because cross reactivities to many other bacteria are reported. However, they were observed together with the 90/93 kDa surface protein, a highly specific borrelial protein.

A broad band pattern between 60 and 90/93 kDa is a support for the diagnosis of chronic Lyme disease and, among the most significant proteins in our study, the 60 and 65 kDa proteins were also observed. The heat shock protein p60 is recognized by cross-reactive antibodies, and according to a study of EUCALB, the inclusion of this protein in the combination for interpreting a Western blot caused slightly superior reactivities.²³

Other highly specific borrelial proteins revealed on blots are the 34 (OspB), 31 (OspA), 30 and 22/24 (OspC) kDa proteins. The number of sera that reacted with these proteins was lower, but the immunoresponse to OspA is rare. The development of antibody reactivity occurs towards the beginning of prolonged arthritic episodes. OspC is the best and earliest marker for early Lyme disease and it is especially detected in IgM blots.²⁴

The most reliable bands for the detection of antibodies against *B. burgdorferi* B31 by IgG Western blot in European patients were those representing the 93, 39, 34, and 23-kDa proteins.²⁵ These proteins were also detected in the Cuban sera studied, except p39.

In conclusion, the serological data indicated that the agent of Lyme borreliosis circulates with a relatively low prevalence in the population under study. These findings represent further data to support the previous serological findings⁶⁻⁸ suggesting the presence of this borreliosis in Cuba, and emphasizing the epidemiological alert for the national health authorities.

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Conflict of interest

All authors declare to have no conflict of interest.

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