INTERNATIONAL JOURNAL OF IMMUNOPATHOLOGY AND PHARMACOLOGY Vol. 22, no. 2, 537-541 (2009)

LETTER TO THE EDITOR

DETECTION OF DIFFERENT *BORRELIA BURGDORFERI* GENOSPECIES IN SERUM OF PEOPLE WITH DIFFERENT OCCUPATIONAL RISKS: SHORT REPORT

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Received November 3, 2008 – Accepted March 16, 2009

This study is aimed at applying a previously described PCR-based method to detect *B. burgdorferi* sensu lato and different *Borrelia* genospecies in total DNA preparations of serum samples collected from people with different occupational risks for tick bite and with serological evidence of borreliosis. Among the seropositive samples, the PCR for *B. burgdorferi* confirmed the positivity in 65% of the forestry workers and in 60% of the subjects living in the same area. None of the seronegative subjects belonging to the control group showed the presence of *B. burgdorferi* sensu lato DNA. Results on genospecies distribution show that *B. afzelii* was the predominant species, followed by *B. garinii* and finally by *B. valaisiana*.

Lyme borreliosis (LB) is the most common vector-borne disease caused by *Borrelia burgdorferi* sensu lato (s.l.) complex, transmitted by ticks of the genus *Ixodes*. LB is common among people occupationally exposed to tick bites, including forestry workers, farmers, veterinarians, military recruits, and outdoor workers in general (1-2). Populations with a high risk of infection have a prevalence of *B. burgdorferi* antibodies greater than other populations (13-47% vs. 2.5-10%) (3). The circulation of *B. burgdorferi* s.l. is strictly correlated with the presence of infected ticks. As previously described (4), infected ticks are frequently found in the region of Lazio, central Italy.

We have previously described a simple, easy-to-perform and reliable protocol for the identification of *Borrelia* from human serum samples by polymerase chain reaction (PCR) (5). The present study is aimed at applying a previously described PCR-based method to detect *B. burgdorferi* s.l. and different *Borrelia* genospecies in serum samples of people with different occupational risks collected in the Lazio region.

At present, the distribution of *B. burgdorferi* s.l. genospecies derives mainly from the analysis of *I. ricinus* vectors (6-7), while few data are available on the presence of *B. burgdorferi* s.l. genospecies in human hosts.

MATERIALS AND METHODS

Serum samples

A total of 60 human blood samples were obtained by

Key words: Borrelia burgdorferi s.l., genospecies, PCR, occupational risk

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0394-6320 (2009)

the Department of Occupational Medicine of ISPESL (Istituto Superiore per la Prevenzione e la Sicurezza del Lavoro). All samples were collected from people living in the Lazio region (Italy). Written informed consent was obtained from all participants who agreed to be enrolled in the study. A questionnaire was compiled by all subjects to obtain information on age, place of residence and work, numbers of hours spent outdoors for work-related and/or leisure activities and for the appearance of symptoms reminiscent of Lyme disease. Also, the frequency of tick bite cases was assessed.

Blood samples were collected from people living in the same area, without Borreliosis symptoms, but all with serological evidence of Borreliosis detected by Elisa and Western blot. Elisa tests were considered negative, positive or borderline when values were lower than 20, higher than 24, or between 20 and 24 U/ml, respectively, as suggested by the manufacturer. Western blot IgM test was considered positive for the presence of 1/4 significant bands: 25 kDa (OspC), 39 kDa (BmpA), 21 kDa, and 41-kDa (Fla). Western Blot IgG test was considered positive for the presence of 2/13 significant bands: 18 kDa, 21 kDa, 25 kDa (OspC), 29 kDa (OspD), 30 kDa, 31 kDa (OspA), 34 kDa (OspB), 39 kDa (BmpA), 41 kDa (Fla), 45 kDa, 58 kDa, 66 kDa, and 93 kDa.

Of the 60 blood samples, 20 (group A) were from seropositive forestry workers (16 males and four females); the mean age was 37.8 years. The duration of employment as a forestry worker varied from 2 to 20 years. Seven of them (35%) recalled a tick bite history and none had clinical signs of active infection at the time blood was taken. Other 20 (group B) blood samples were from seropositive subjects living in the same area as the forestry workers, but with different jobs generally considered not at occupational risk for tick bite. They consisted of 20 males; the mean age was 38.0 years. Finally an additional group of 20 (group C) seronegative subjects were included as control group: ten forestry workers and ten subjects not at risk.

All sera, after serological testing, were aliquoted and stored at -80°C until DNA extraction.

DNA extraction and amplification

The *Borrelia* total DNA extraction method was applied to all sera collected, as previously described (5). PCR experiments were performed using 16S rRNA gene specific primers designed for *B. burgdorferi* s.l. (5'-ATG CAC ACT TGG TGT TAA CTA GAC TTA TCA CCG GCA GTC TTA-3'), *B. burgdorferi* sensu stricto (s.s.) (5'-GGG ATG TAG CAA TAC ATT CAT ATA GTT TCC AAC ATA G-3'), *B. garinii* (5'-GGG ATG TAG CAA TAC ATC TAT ATA GTT TCC AAC ATA GT-3'), *B. afzelii* (5'-GCA TGC AAG TCA AAC GGA ATA TAG TTT CCA ACA TAG C-3') and *B. valaisiana* (5'-GCA AGT CAA ACG GGA TGT AGT GTA TTT TAT GCA TAG ACT TAT ATG-3') (8-9). Briefly, PCR reactions were performed in a volume of 25 µl containing 12.5 pmol of appropriate primer set and 1 µl of bacterial total DNA preparation. PCR amplifications were performed using a Perkin-Elmer Cetus thermocycler according to previous described protocol (5). Amplicons were visualized after electrophoresis in 1% agarose in TAE buffer [40 mM Tris-acetate, 2 mM EDTA (pH 8.5)] containing ethidium bromide at 0.5 pgml-1.

RESULTS

We analyzed a total of three groups. Seropositive samples from subjects exposed (group A) to tick bite, seropositive samples from subjects not exposed to tick-bite (group B) and seronegative samples (group C) as control group.

When blood samples of the group A were analysed by Elisa and Western Blot, 19 showed the presence of significant levels of IgM ($\geq$ 24U/ml) and one the presence of IgG ($\geq$ 24U/ml) specific antibodies against *B. burgdorferi.* Of the other 20 seropositive samples (group B), 18 had IgM and 2 had IgG antibodies against *B. burgdorferi* (Table I).

We applied the new PCR method (5) for the detection of the *B. burgdorferi* s.l. DNA in subjects who did not present clinical signs and symptoms of Borreliosis and with different occupational risk.

Among the samples with anti-*B. burgdorferi* antibodies, the PCR confirmed positive reaction for *B. burgdorferi* s.l. genoma in 13/20 (65%) in group A, 12/20 (60%) for group B, and none in group C (Table II). Our results indicate that *B. afzelii* was the predominant species, followed by *B. garinii,* and finally by *B. valaisiana* (Fig. 1). *B. afzelii* alone or in association was detected in 77% of the group A and in 66.6% of the group B, *B. garinii* in 38.8% of the group A and in 33.3% of the group B and *B. valaisiana* in 30.7% of the group A and in 25% of the group B. *B. burgdorferi* s.s. was not found. Mixed infections by two species were detected in 46% of the group A and in 25% of the group B. Individual ticks may be infected with multiple genospecies of *B. burgdorferi* s.l. Mixed genospecies carriage in individual ticks unravels important clues on the biology and ecology of *Borrelia.*

Table I. *IgG and IgM B. burgdorferi Western blot, frequency of the antigenic bands.*

Population studied (+/ Tot)	p83/ 100 kDa	p58 kDa	p41 kDa	p34 kDa	p30 kDa	p29 kDa	p25 kDa	p21 kDa	p18 kDa
Group A IgM (19/20) IgG (1/20)	- -	- -	12 -	2 -	1 -	- -	11 1	7 1	- -
Group B IgM (18/20) IgG (2/20)	1 -	- 1	4 1	- -	1 1	1 -	15 1	11 1	1 -

Group A: seropositive forestry workers; Group B: seropositive subjects living in the same area

Table II. *Prevalence of B.burgdorferi s.l. DNA in the 3 populations studied.*

Population studied	N.° PCR positive/N.° tested	Percentage positive
Group A	13/20	65%
Group B	12/20	60%
Group C	0/20	0%

Group A: seropositive forestry workers; Group B: seropositive living in the same area; Group C: seronegative

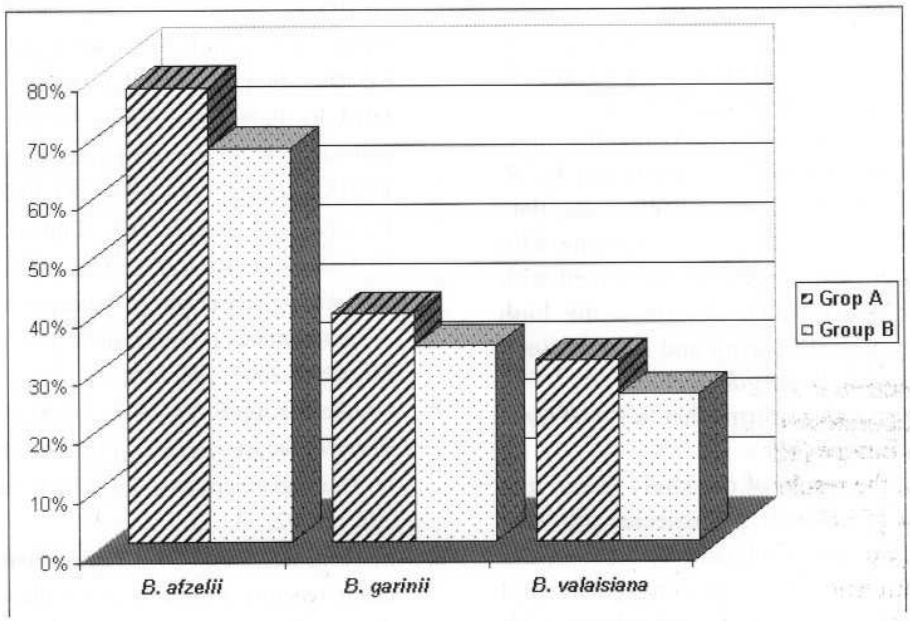

Fig. 1. *Distribution of B. burgdorferi s.l. genospecies in serum samples of PCR positive group A and group B.*

DISCUSSION

In this study we applied a previously described PCR method to detect *B. burgdorferi* s.l. and different *Borrelia* genospecies in serum samples. The results presented show that our protocol once again has proven useful to detect and to differentiate the main species of *B. burgdorferi* s.l. Moreover, our results suggest that the use of PCR has probably avoided the false positive identification that sometimes occurs with serological tests (10). The high percentage of IgM antibodies for group A and B (>90%) likely indicates concurrent infection by *B. burgdorferi,* in spite of the absence of clinical signs and symptoms of infection. This is in agreement with the finding that not all infected individuals develop the clinical symptoms of the disease and that latent infections may often be misdiagnosed (11). Moreover, these results are in agreement with those of Guy & Stanek (1991), underlining that the PCR method can contribute to the reliability of diagnosis during the early stages of infection (12).

At least three distinct species of *B. burgdorferi* s.l. have been described as human pathogen: *B. afzelii*, *B. garinii* and *B. burgdorferi* s.s. Other *Borrelia* genospecies, such as *B. valaisiana* have been isolated from ticks in Europe and are involved in human Lyme borreliosis (13). Each species is correlated with distinct clinical manifestations: *B. afzelii* is known to be associated with skin manifestations, *B. garinii* is mainly found in neuroborreliosis and *B. burgdorferi* s.s with arthritic symptoms (14).

This study shows *B. afzelii* as being the most frequent species followed by *B. garinii* and by *B. valaisiana.* These results not only confirm the data we previously obtained by analyzing patients with Lyme borreliosis (5), but are also in agreement with several studies (15-18) which described the high prevalence of *B. afzelii, B. garinii* and *B. valaisiana* in European countries. *B. burgdorferi* s.s. DNA was not recovered, this result confirms that this species is relatively rare in Europe (19).

In conclusion, the results of our study reveal that the identification of different genospecies provides essential information for clinicians in view of the demonstrated correlation between genospecies and clinical syndromes. Moreover, in accordance with other studies (20), our preliminary results support the hypothesis that serological testing compared to PCR may overestimate the diagnosis of LB.

ACKNOWLEDGEMENTS

This work was supported by Faculty 60% funds granted to I.S.

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