PREVALENCE OF IgG ANTIBODIES AGAINST *BORRELIA BURGDORFERI* S.L. AND *EHRLICHIA PHAGOCYTOPHILA* IN SERA OF PATIENTS PRESENTING SYMPTOMS OF LYME DISEASE IN A CENTRAL REGION OF ITALY

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Received December 3, 2000 - Accepted February 11, 2002

The aim of this study was to evaluate the prevalence (seroprevalence) of antibodies against *Borrelia burgdorferi* and *Ehrlichia phagocytophila* among patients resident in Lazio, a region of central Italy. Of a sample of 1,050 patients, which presented clinical manifestations related to Lyme disease, 34 (3.2%) were *Borrelia*-seropositive (Lyme index value ≥ 1.2). The sera of 25 out of the 34 patients that were *Borrelia*-positive were also analysed for the presence of antibodies against *E. phagocytophila* and 3 (12%) were found *Ehrlichia*-positive (titres >1:64). No *Ehrlichia*–positive samples were found among sera of 250 *Borrelia*-negative patients. Since both *B. burgdorferi* s.l. and *Ehrlichia* species share the same tick vector (*Ixodes ricinus*), our results indicate that concurrent transmission of these microbial pathogens might have been occurred among the patients included in this study.

Ehrlichioses are emerging tick-transmitted infections caused by gram-negative obligate intracellular bacteria, belonging to the genus *Ehrlichia*, that infect leukocytes or platelets of various mammalian species. The first case of human disease (human monocytic ehrlichiosis; HME) caused by a monocytotropic *Ehrlichia* species (*Ehrlichia chaffeensis*) was described in 1987, while the first case of human granulocytic ehrlichiosis (HGE), which is caused by *Ehrlichia phagocytophila*, was reported in 1994 (1, 2). In Europe, the first cases of HME and HGE have been reported in Portugal and in Slovenia, respectively (3, 4). To our knowledge no clinically-referred cases of HME or HGE have as yet been reported in Italy.

Ticks are vectors that transmit ehrlichiosis and Lyme borreliosis and *Ixodes ricinus*, the high prevalence tick in the geographical region considered in this study, is known to transmit *Borrelia burgdorferi* s.l. and *E. phagocytophila* (5). Since parts of Italy have been considered endemic for Lyme borreliosis (6), and coinfection with *B. burgdorferi* and granulocytotropic ehrlichiae was recently documented (7), we decided to undertake a seroepidemiologic study to determine i) the prevalence of antibodies against *B. burgdorferi* among patients resident in Lazio, a region of central Italy, presenting clinical manifestations related to the Lyme borreliosis, and ii) to search for *B. burgdorferi-E. phagocytophila* double-positive patients to estimate putative double infections.

MATERIALS AND METHODS

Serum samples

Serum samples were analysed at the Dipartimento di Scienze di Sanità Pubblica, Università "La Sapienza" and at the Istituto di Microbiologia, of the Università Cattolica del Sacro Cuore of Rome. A total of 1,050

Key words: Ehrlichia phagocytophila, Borrelia burgdorferi, Ixodes ricinus

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0394-6320 (2002)

serum samples were collected from patients (range 3 to 76 years of age) presenting clinical manifestations typical of Lyme borreliosis, including common clinical manifestations following the bite of a tick (erythema migrans, fever, malaise, fatigue, skin rash, and so on), or later stages of Lyme disease (severe arthritic, neurologic and cardiac manifestations). As a control group, 330 serum samples from healthy individuals (blood donors) also resident in the same geographical area and that did not report previous tick-bites or symptoms compatible with Lyme disease were also included in this study.

B. burgdorferi *ELISA and IFA for anti-HGE antibody detection*

Serum samples were tested for IgG antibodies to *B. burgdorferi* by a commercial standard ELISA kits (MarDx, Diagnostic Inc., San Diego, CA) according to the manufacturer's instructions. Sera showing Lyme index values (LIV) ≥ 1.2 were considered positive, as recommended by the manufacturer. IgG-positive serum samples were confirmed by Western blot with a commercially available kit (MarDx, Diagnostic Inc., San Diego, CA). The criteria used to interpret the results of Western blot experiments were as described by Hauser *et al.* (8). Sera that were positive, together with a representative group of 250 serum samples that were found negative for *B. burgdorferi*, were further tested for IgG antibodies to *Ehrlichia* by a polyvalent IFA using a commercial kit (MRL Diagnostics, USA). This assay utilises HL-60 cells infected with a HGE-1 human isolate and was performed in accordance with the manufacturer's procedure. Briefly, 25 µl aliquots of each serum sample, as well of IgG-positive and IgG-negative controls, were diluted in PBS and placed on a slide-well plate, in contact with the substrate. Slides were incubated in a humid chamber for 30 minutes at 37°C. After washing, 25 µl of IgG-conjugate were added to each slide-well and incubation was continued for further for 30 min. Wells were washed, dried, mounted and examined using fluorescence microscopy. Positive and negative-controls were included in each run. Sera showing titres ≥1:64 were considered positive.

RESULTS

Serum samples serology

Of the 1,050 serum samples of patients included in this study, presenting clinical manifestations typical of Lyme borreliosis, 34 (3.2%) were found positive for IgG antibodies to *B. burgdorferi*, by the ELISA test employed in this study (Tab. I). Among control blood donors, 7 sera were found positive (1.5%). Positivity was confirmed in all cases by Western blot. Of the 34 *B. burgdorferi*-positive sera, 25 were available to search for antibodies to *Ehrlichia phagocytophila*. 3 (12%) out of the 25 *B. burgdorferi*-positive sera analysed were found also to be positive also for *E. phagocytophila* IgG antibodies (titres >1:64) (Tab. I). Of the three patients found positive for antibodies to *E. phagocytophila,* two, a 45-year-old man presenting neurological symptoms and a 50-year-old man with arthralgias, could not remember a tick bite prior the onset of their illness. The third patient was a 7-year-old child, presenting recurrent fever and neurological symptoms, who recalled having sustained a tick bite on his leg few months before the onset of the illness.

Tab. I. *Antibody titers to* B. burgdorferi *s.l. and to* E. phagocytophila *in patients with manifestations related to Lyme borreliosis.*

No. of serum samples[a]	Age Range	*B. burgdorferi*		*E. phagocytophila*		*E. phagocytophila*[e]
		IgG+[b]	LIV[c]	IgG+[d]	titre	IgG+[f]
1,050	3 - 76	34 (3.2)	>1.2	3 (12)	>1:64	0

[a] From patients presenting clinical manifestations related to Lyme borreliosis.
[b] Number (%) of serum samples found positive for IgG to *B. burgdorferi.*
[c] LIV, Lyme index value.
[d] Number (%) of serum samples found positive for IgG to *E. phagocytophila*; only 25, out of the 34 IgG positive for *B. burgdorferi* were tested. Antibody titre is reciprocal of the serum dilution.
[e] Analysis of IgG titre to *E. phagocytophila* among 250 serum samples found negative for IgG to *B. burgdorferi.*
[f] Number of serum samples found positive to *E. phagocytophila.*

None of 250 serum samples, chosen among patients found negative for *B. burgdorferi* antibodies, were found positive for *E. phagocytophila* (anti-HGE antibody detection; Tab. I), indicating that probably tick-mediated transmission of *E. phagocytophila* alone must occur at a very low-frequency among patients living in the geographical area chosen for this study.

DISCUSSION

Epidemiological studies on the diffusion of *Ehrlichia* in Italy are scanty owing to the fact that the interest regarding these agents and the diseases induced by them is very recent. Until now, no cases of the disease in humans have been reported in Italy, even if serological studies suggested that human infections by *Ehrlichia* species may not be so rare.

Serological surveys, using *E. phagocytophila* as antigen, have been conducted among patients living in Italy and in other European countries (9-11). A study conducted in 1996 revealed the presence of significant levels of antibodies to *E. phagocytophila* in about 20% of forestry workers (against 2% of a control group) working in the alpine and sub-alpine zones of north-eastern Italy (12). Moreover, other studies, carried out with different patients categories, in the same geographical area, reported 6.3% and 8.6% positivity (13-14).

Recently, we have reported the prevalence of antibodies to *E. phagocytophila* in serum samples of park-workers of the Parco Nazionale d'Abruzzo, the largest Italian National Park. Interestingly, while the serological positivity among this group of workers was 4.5%, that of the inhabitants of the same Park were up to 8% (15). In Molise, a region of southern Italy, the serological positivity was reported as high as 1.1% (16).

These different antibody values among subjects presenting symptoms of Lyme borreliosis living in different areas are most probably due to the presence of different extension of green areas and of parks, and thus of the related numbers of ticks of the species *I. ricinus* which are known to transmit the diseaseas. Moreover, the concentration of specific antibodies (the antibody titre) in the blood of patients, at the time of clinical presentation, might not represent the real incidence of these infections, that might be also further underestimated if we consider that they are often asymptomatic (17).

Concerning the *Ehrlichia* species hosted by the tick *I. ricinus*, the presence of *E. phagocytophila* and of HGE-like *Ehrlichia* in ticks inhabiting central and north-eastern Italy has been demonstrated (18), and concurrent hosting of *B. burgdorferi* s.l. and *E. phagocytophila* has also been shown (18-19). In this respect, the percentage of cases of patients showing seropositivity to both bacterial species shows a great variability (20-22), ranging from a 33.3% in a group of forest service workers in Italy (13), to 3.2% in a Swedish population (5). In this study, of 1,050 sera tested of patients presenting symptoms of Lyme borreliosis, 34 (3.2%) were found positive for IgG antibodies to *B. burgdorferi* and, of these, 25 were searched for antibodies to *E. phagocytophila.* The results obtained indicated that 3 (12%) out of the 25 sera tested were found to be positive also to *E. phagocytophila*, while 250 sera, randomly chosen among the 1,050 that were negative for *B. burgdorferi*, were negative for *E. phagocytophila* antibodies (Tab. I).

The cases of double seropositivity, detected in this work, and the presence of both *B. burgdorferi* s.l. and *Ehrlichia* in the vector *I. ricinus* (19), suggest the presence of double infections, albeit at a low frequency, in the sample population chosen for this study. Since different considerations might influence the interpretation of studies centered on the simple determination of antibody titres, including previous exposure, simultaneous seroconversion or seroreactivity, more studies are surely needed to assess with more precision the incidence of these tick-transmitted infections, and to elucidate the immunological basis leading to individual sensibility towards these microbial pathogens.

ACKNOWLEDGEMENTS

This work was supported by Faculty 60% funds granted to M.d.P., and in part by MURST PRIN research project "Effettori di virulenza in patogeni enterici: caratteristiche e studio delle loro interazioni" granted to M.N.

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