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Coexistence of *Ehrlichia phagocytophila* and *Borrelia burgdorferi* Sensu Lato in *Ixodes ricinus* Ticks from Italy as Determined by 16S rRNA Gene Sequencing

Human ehrlichiosis is a newly recognized rickettsial disease, first described in 1986 (5) in the United States. The first European case of ehrlichiosis, diagnosed on a serological and clinical basis, was reported in Portugal in 1991 (7). Furthermore, serological surveys recently conducted in Switzerland and the United Kingdom have shown the presence of antibodies to *Ehrlichia phagocytophila* in 5 to 7% of subjects bitten by ticks (1, 11). Recently, a case of human granulocytic ehrlichiosis (HGE) infection was reported in Slovenia (10). The species involved in animal ehrlichiosis in Europe are *E. canis*, a monocytic ehrlichia, and granulocytic ehrlichiae of the *E. phagocytophila* genogroup (4, 9). The vectors for ehrlichiae have been identified as *Rhipicephalus sanguineus* for *E. canis* and *Ixodes ricinus* for *E. phagocytophila*. Some work, based on PCR, on the detection of granulocytic ehrlichiae in Swedish ticks has been done, and indeed, a 16S rRNA gene sequence identical to that of the HGE agent has been found in one tick (13). However, on the whole, very little is known about the animal reservoir and the ecology of ehrlichiae throughout Europe. It has been supposed, on the basis of the coexistence of antibodies to tick-borne pathogens of ehrlichiosis and Lyme borreliosis (LB) in human sera, that the same ixodid ticks can be coinfected by *Borrelia burgdorferi* and *Ehrlichia* (6). Furthermore, the geographic distribution of HGE in the United States usually overlaps that of LB in those territories where the same ticks are present (12).

In Italy *Ehrlichia* infections are present in dogs (*E. canis*) and horses (8), but neither human cases of clinically documented HGE nor any evidence of the organism in vector ticks has been reported. Since our investigations deal with the detection of *B. burgdorferi* in *I. ricinus*, in different areas of Italy, we looked for the presence of *Ehrlichia* in samples of ticks collected in an area of central Italy, where a certain prevalence of *B. burgdorferi* infection was detected (3). During springtime, ticks were collected by flagging vegetation in the Manziana Park, a recreational area in the Lazio region. DNAs from 86 ticks (84 nymphs and 2 adults) were extracted as described previously and analyzed for the presence of *B. burgdorferi* (2, 3). For *Ehrlichia*, a PCR-based assay, which has been developed for the specific demonstration of *E. phagocytophila* genogroup ehrlichiae in animal blood, was employed (9). In this PCR, two primers targeting sequences of the 16S rRNA gene are used. These primers specifically amplify DNA from *E. phagocytophila*, *E. equi*, and a newly described Swedish *Ehrlichia* species, which has a 16S rRNA sequence identical to that of the HGE agent (4). The ehrlichiae of the *E. phagocytophila* genogroup (including the HGE agent) have very similar 16S rRNA sequences, but close to the 5′ end a few nucleotide differences exist. With the PCR assay described here, this region is amplified, and by subsequent sequence analysis of the PCR products the identity of the isolated ehrlichia can be established (9).

Of the 86 tick DNA samples prepared, 21 (all nymphs) were amplified by the *Ehrlichia* PCR, giving a positivity value of 24.4% (Fig. 1B). Of the same tick DNAs, 19.8% were PCR positive for *B. burgdorferi* sensu lato only (Fig. 1A) and 8.14% were positive for both organisms. The amplicons of three *Ehrlichia* PCR-positive samples were analyzed by solid-phase DNA sequencing of part of the 16S rRNA gene (4, 9). Segments of 400 to 500 bp were analyzed, and the sequences of all three samples were found to correspond to the sequence of *E. phagocytophila*. This indicates that the Italian *I. ricinus* ticks can be coinfected with both *E. phagocytophila* and *B. burgdorferi.*

This is the first report of coexistence of *B. burgdorferi* sensu lato and granulocytic ehrlichiae in European ticks. Therefore, the same vector might act as a potential source of both human ehrlichiosis and LB. Bearing in mind the report of HGE in Slovenia (10), more attention should be paid to the presence of clinical symptoms of human ehrlichiosis in people exposed to *I. ricinus* bites in those areas where LB is endemic.

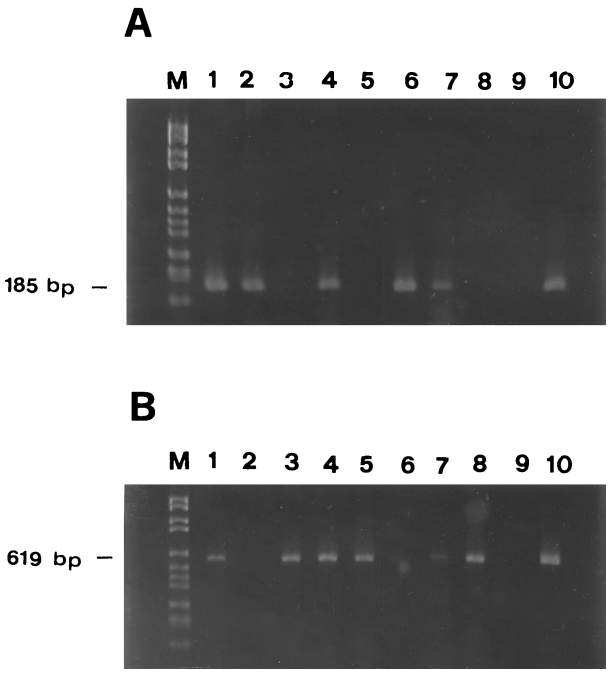

FIG. 1. Results of PCR assay for detection of *B. burgdorferi* (A) and *Ehrlichia* species (B) in the same samples of *I. ricinus*. Each lane contains a different sample. DNAs in lanes 1, 4, and 7 were amplified by primers for both *Ehrlichia* and *B. burgdorferi*. Lanes 9, negative controls; lanes 10, positive controls. M, molecular size marker.

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