with enterococcal antigen, the bands produced were quite weak, and the pattern of bands was different from that seen with sera from patients with enterococcal infections (Figure 2). Since we selected these sera randomly without knowledge of the patients' clinical conditions, it is possible that they were infected with enterococci or other gram-positive organisms and could have had antibodies that cross-reacted with enterococcal antigens.

In conclusion, this pilot study suggests that Western blot may aid in the diagnosis of enterococcal infections. Patients with enterococcal endocarditis can often be distinguished from those with other enterococcal infections by a strongly reactive Western blot in which specific bands are present. Likewise, patients with bacteremia and deep-seated infections may sometimes be distinguished from those with bacteremia alone. However, the presence of some cross-reactivity with other gram-positive infections suggests that a more specific antigen preparation might be more useful in the serologic diagnosis of enterococcal infections.

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Serological Evidence of Human Granulocytic Ehrlichiosis in Norway

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Human granulocytic ehrlichiosis was first described in 1994. This tick-transmitted illness is increasingly recognized in the USA as well as in

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Table 1: Characteristics and immunoserologic reactivities of study group patients and controls with elevated antibody titers to *Ehrlichia equi*.

Patient subject no.	Group	Sex	Age (years)	*E. equi* titer by IFA	Lyme EIA
2	study	M	64	160	positive
18	study	F	58	80	positive
34	study	M	61	160	positive
37	study	M	46	80	positive
47	study	M	65	160	positive
54	study	F	68	80	positive
77	control	M	49	80	negative

EIA, enzyme immunoassay; IFA, indirect immunofluorescent assay.

Europe in areas where *Ixodes* ticks and Lyme borreliosis are endemic. Blood samples from 58 Norwegian patients with physician-diagnosed Lyme borreliosis were examined for the presence of antibodies to *Ehrlichia equi*, a surrogate marker of the agent of human granulocytic ehrlichiosis. The results indicated that 10.2% of the patients may have been co-infected with human granulocytic ehrlichiosis and Lyme borreliosis. Human granulocytic ehrlichiosis appears to be established in southern Norway.

Human granulocytic ehrlichiosis (HGE) has been diagnosed in the USA since 1990 (1–5). Recent serologic studies of serum samples from patients with laboratory-confirmed Lyme disease have indicated that HGE also may occur in Europe, including Switzerland (6) and the UK (7). Human granulocytic ehrlichiosis-endemic areas appear to overlap with the areas where *Ixodes* ticks and Lyme borreliosis are commonly found (8).

Materials and Methods. *Ixodes ricinus* ticks are found endemically in southern Scandinavia, and Lyme borreliosis is the most common tick-borne illness diagnosed in coastal southern Norway. We wanted to look for antibodies to the agent of HGE in patients with a presumed recent *Ixodes ricinus* tick bite, evidenced by physician-diagnosed and serologically-confirmed active Lyme borreliosis. Sixty serum samples from 58 patients with clinical Lyme borreliosis (study group) were tested retrospectively for antibodies to *Ehrlichia equi*, a surrogate marker for the agent of HGE. All patients resided in the southern region of Telemark County, Norway, and all samples were collected between 1 March 1993 and 3 November 1995. No samples were obtained in January, February, or May. Fifty-one additional serum samples from random blood donors living in the same geographical area were used as controls. No epidemiological or clinical information or suitable blood samples for polymerase chain reaction analysis or light microscopic blood smear evaluation for detection of typical neutrophilic morulae (1) were available from any of the patients or controls.

The study group patients had been diagnosed previously with acute Lyme borreliosis by their own physicians, based on clinical evaluation and a positive test for antibodies to flagellin by the IDEIA *Borrelia burgdorferi* IgG/IgM Test Kit (Dako Diagnostics, UK). Serum samples from all study group patients and controls were also tested by an enzyme immunoassay (EIA) for *Borrelia burgdorferi* immunoglobulin G (IgG) and IgM antibodies (Lymelisa II Test Kit; Biowhittaker, USA).

A modification of the indirect immunofluorescent antibody (IFA) method using *Ehrlichia equi* (courtesy of J. Madigan, University of California, Davis, USA) as described previously was used to test all serum samples from the control group and the study group (1). Serum samples positive for *Ehrlichia equi* were also tested for antibodies to *Ehrlichia chaffeensis* (antigen kindly provided by J. Dawson, Centers for Disease Control and Prevention, Atlanta, USA) (1). Values of $\geq$ 1:80 were considered positive since previous studies as well as our own experience have shown that nonspecific reactivity is likely to occur in less diluted serum (1). Immunofluorescent antibody titer values were expressed as the reciprocal of the representative serum dilution.

Values were compared using Fisher's exact test, the Mann-Whitney and Wilcoxon rank sum test, or the chi-square test with Pearson's correlation

coefficient. Odds ratios were estimated using Epi-Info Version 6 software (CDC, USA), and risk was calculated by the Mantel-Haenszel method. P values of ≤ 0.05 were considered statistically significant.

Results and Discussion. The study group consisted of 31 females and 27 males with mean ages of 49.7 years (standard deviation 23.0) and 47.1 years (SD 18.6), respectively. There were 20 females and 31 males in the control group; their mean ages were 41.5 years (SD 9.2) and 41.2 years (SD 11.0), respectively. Although the controls were younger than the patients in the study group ($p = 0.028$), there was no significant difference in terms of gender between the study group patients and controls ($p = 0.14$).

Four males and two females in the study group had elevated antibody titers to *Ehrlichia equi* (Table 1). The absolute *Ehrlichia equi* titer values were low, ranging from 80 to 160. Patient 18 had a stationary IFA titer of 80 in two separate samples taken two weeks apart. A 49-year-old male control subject (no. 77) also had an *Ehrlichia equi* IFA titer of 80, but he did not have antibodies to *Borrelia burgdorferi* (Table 1). None of the positive serum samples reacted with *Ehrlichia chaffeensis*. Patients in the study group were found to have a relative risk of 5.28 × (95% confidence interval 0.66–42.37) for having had HGE when compared to the control group subjects. Each of the study group patients lived in a separate town within a region known to be highly endemic for *Ixodes ricinus* ticks, but the single control subject who had antibodies to *Ehrlichia equi* lived outside the endemic region. Only three of the control subjects (6%) had antibodies to *Borrelia burgdorferi*, in contrast to the study group patients, all of whom were seropositive; this difference was statistically significant ($p < 0.001$).

The incidence and areas of distribution of HGE are largely unknown. Reports on HGE published to date suggest that transmission of the agent of HGE likely involves a tick vector, members of the *Ixodes ricinus* complex being the most probable candidates (3, 9–11). Lyme borreliosis in Norway is acquired through the bite of an infected *Ixodes ricinus* tick. This tick species is endemic in the coastal (southern) regions of Telemark County (12). The estimated seroprevalence of Lyme borreliosis was 6% (3 of 51) in our control population.

Previous surveys of patients with Lyme borreliosis from Switzerland (6), the UK (7), and Sweden (J. S. Dumler, 1996, unpublished data) have suggested the presence in these countries of an agent that reacts serologically with *Ehrlichia equi*. Coinfections with *Borrelia burgdorferi* and granulocytic *Ehrlichia* spp. may occur in 5.2 to 12% of patients with Lyme borreliosis (10, 13, 14). The most likely vector of HGE in Europe is *Ixodes ricinus*. Thus, it is not surprising that six of the 58 (10.2%) patients from Telemark County with Lyme borreliosis also demonstrated serologic evidence of exposure to *Ehrlichia equi* or an agent closely related to it.

The 10.2% HGE prevalence rate observed in our study group closely resembles the frequency noted in investigations of patients from Minnesota and Wisconsin (9, 14, 15). We have previously shown that IFA titer values of ≥ 80 reliably separate HGE-seroreactive from -nonreactive patients (1, 15). Only one of the healthy controls (no. 77) had antibodies to *Ehrlichia equi*, in contrast to six of the study group patients. This difference nearly reached statistical significance ($p = 0.079$). Magnarelli et al. (13) found no false-positive serologic responses to *Ehrlichia equi* in patients who had no prior history of rickettsial diseases. Thus, we think it is unlikely that our patients would have had positive serum samples unless they had been exposed previously to the agent of HGE. Based on the findings in this investigation, patients with Lyme borreliosis were 5.28 times more likely to have had HGE than the control subjects which would support the contention that *Ixodes ricinus* is a likely vector of the agent of HGE.

The mean age of the patients with *Ehrlichia equi* antibodies was 60.3 years (SD 7.8) which is nearly identical to the mean age of patients with HGE in the upper midwest of the USA (15). We are unable to explain why the absolute titer values were relatively low, but this may reflect blood sampling during the early stage of disease (early seroconversion), late convalescent phase following past exposure, or slight antigenic divergence from *Ehrlichia equi* or the agent that causes HGE.

In conclusion, we have shown that antibodies to the agent of HGE or the closely related species *Ehrlichia equi* were present in 10.2% of a patient group with acute Lyme borreliosis in southeast Norway. Human granulocytic ehrlichiosis and Lyme borreliosis may occur as co-infections, since bites by infected ticks in the *Ixodes ricinus* complex may transfer the agents responsible for both illnesses (9, 13, 14). Since HGE can be fatal, we believe that tetracycline or doxycycline, which are active against both bacterial agents should be

considered the preferred antibiotic treatment choices for patients who present with a history of a tick bite and an acute febrile illness consistent with Lyme borreliosis, HGE, or both.

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Comparison of the Indirect Immunofluorescent Antibody Test and the Direct Agglutination Test for Serodiagnosis of Visceral Leishmaniasis in HIV-Infected Subjects

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One hundred subjects positive for anti-human immunodeficiency virus (HIV) antibodies were tested for anti-*Leishmania* antibodies by the indirect immunofluorescent antibody test (IFAT) and the direct agglutination test (DAT). Subjects were subsequently followed for two years to monitor the onset of visceral leishmaniasis. Fifteen subjects were positive for anti-*Leishmania* antibodies in either one or both tests. Eleven were positive only by IFAT, one only by DAT, and three by both tests. During the two-year follow-up period, nine subjects developed visceral leishmaniasis; of these, six were serologically positive, four by IFAT alone and two by both tests. The results indicate that IFAT and DAT have a similar specificity but that IFAT has a higher sensitivity and a greater diagnostic significance.

Serological tests for the diagnosis of leishmaniasis present difficulties when attempting to reliably establish disease status, whether acute, subacute, or asymptomatic. This is due to the levels of

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