incubation time can be made on the basis of the data presented.

We conclude that a combination of the membrane filter method and culture on CCDA will result in maximal recovery of *Campylobacter* spp.

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Three-Year Study of Antibody to *Borrelia burgdorferi* in Southern Spain

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The prevalence of anti-*Borrelia burgdorferi* antibodies was studied in Granada, Spain, between January 1991 and November 1993 in 354 patients with suspected Lyme disease (group 1); in 50 patients either with syphilis (n = 32) or without syphilis but with a positive Rapid Plasma Reagin test (n = 18) (group 2); and in 150 healthy subjects (group 3). In addition, intrathecal antibody production was evaluated by EIA in CSF samples obtained from 117 patients in group 1. Anti-*Borrelia burgdorferi* antibodies were detected by EIA in 58 patients (16.4 %) in group 1, 29 (8.2 %) of whom were positive by Western blot. Intrathecal antibody production was detected in one patient. In group 2, 8 (16 %) patients had a positive EIA result, but none of these was confirmed by Western blot. Western blot was negative for all subjects in group 3. The re-

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sults of this study indicate that anti-*Borrelia burgdorferi* antibodies are not uncommon in our area, although Lyme disease is rare.

Lyme disease is a multisystemic disease that affects a variety of organs such as the skin, the heart and the central nervous system (1). It is transmitted to humans via the bite of a tick belonging to the *Ixodes* genera. Since being first identified in 1981 as the causal agent of Lyme disease (2), *Borrelia burgdorferi* has been the focus of numerous studies investigating new methods and diagnostic criteria in order to facilitate the correct diagnosis of this disease. Other studies have aimed at establishing the prevalence of *Borrelia burgdorferi* in various geographical regions. These latter studies could prove useful in the treatment of patients with nondefinitive symptoms or serological results.

The objectives of this study were to evaluate the prevalence of antibodies to *Borrelia burgdorferi* in a defined region of Spain during a three-year period and to determine which clinical manifestations are associated with these antibodies.

Material and Methods. The prevalence of anti-*Borrelia burgdorferi* antibodies was studied in the Granada region of Spain between January 1991 and November 1993. Group 1 of the study population consisted of 354 patients with suspected Lyme disease; group 2, 32 patients with syphilis and 18 patients without syphilis but with a positive Rapid Plasma Reagin test (Becton-Dickinson, USA); and group 3, 150 healthy subjects as controls. Group 2 was included to control the specificity of the techniques. One serum sample from each individual was studied, and a cerebrospinal fluid (CSF) sample was also taken from 117 patients in group 1 to test for neuroborreliosis. Intrathecal antibody production was evaluated by EIA (Pasteur-Sanofi, France) as described previously (3). Criteria used for positivity of clinical borreliosis were those defined by the Centers for Disease Control and Prevention (CDC): erythema, arthritis, lymphocytic meningitis, neuritis, the Guillain-Barré syndrome or intense auriculo-ventricular blockage (4).

Serum samples from all subjects were tested by indirect EIA, using the sonicated *Borrelia burgdorferi* B31 strain (Pasteur-Sanofi) in the solid phase, to determine the presence of specific IgG and IgM antibodies. Serum samples with absorbances equal to or greater than the cut-off point indicated by the manufacturer and also those with an absorbance up to 10 % lower than this value were taken as positive and confirmed by Western blot (WB) (MarDx, USA). The WB test was used to study IgG and IgM antibodies, the latter in the presence of anti-IgG (Rheumathic Factor Absorbent, Behring Institute, Germany). The results were expressed using the following criteria: positive, presence of bands 41 or 39 and 31 or 34, or bands 31 and 34; negative, absence of all bands or isolated presence of bands 60, 66 or 41; and equivocal, presence of bands in any combination not described here (5) (Figure 1). Finally, anti-*Treponema pallidum* antibodies were studied in all groups.

Results and Discussion. Fifty-eight of 354 patients (16.4 %) in group 1 were positive for anti-*Borrelia burgdorferi* antibodies, 29 (8.2 %) of whom were positive by WB. The majority of serum samples were positive for IgG antibodies alone, although five samples were positive for IgM antibodies alone and six were positive for both antibody classes. In one patient in whom antibodies were detected by EIA in the CSF, the WB test was equivocal for IgG antibodies (band

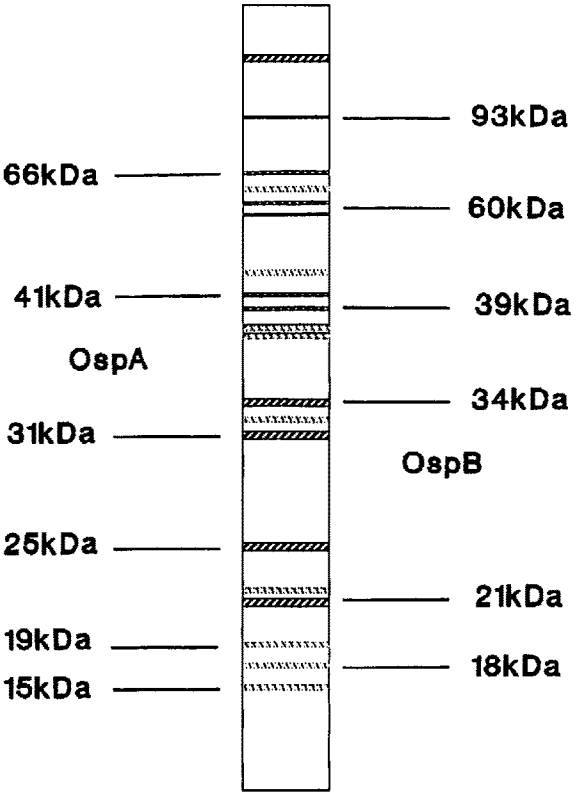

Figure 1: Proteins to *Borrelia burgdorferi* detected by Western blot.

Table 1: Prevalence of antibodies to specific proteins as determined by Western blot in 29 group 1 patients positive by EIA.

Western blot result	Percentage of patients with antibodies to specific proteins												
	93 kDa	75 kDa	66 kDa	60 kDa	41 kDa	39 kDa	34 kDa	31 kDa	29 kDa	25 kDa	21 kDa	18 kDa	15 kDa
IgG positive (n = 18)	44.4	0	66.7	66.7	94.4	44.4	66.7	44.4	44.4	16.6	44.4	11.1	22.2
IgM positive (n = 5)	0	0	40	20	40	20	100	60	0	0	40	40	0
IgG and IgM positive (n = 6)	16.6	16.6	100	83.3	100	83.3	100	66.7	16.6	33.3	50	0	0

Table 2: Prevalence of antibodies to *Borrelia burgdorferi* in Granada, Spain.

	Sample	No. tested	No. (%) positive by EIA	No. (%) positive by Western blot
Group 1	serum	354	58 (16.4)	29 (8.2)
	CSF	117	1 (0.9)	0
Group 2	serum	50	8 (16)	0
Group 3	serum	150	1 (0.67)	0

34) and negative for IgM antibodies. Intrathecal antibody production was revealed by the following result: albumin concentration in CSF/albumin concentration in serum = 3.8×10^{-3} (breakpoint: 7.5×10^{-3}).

Bands occurring with the greatest frequency in patients positive for IgG antibodies by WB were 41 (94.45 %), 66 (66.7 %), 60 (66.7 %) and 34 (66.7 %) (Table 1). Of these patients, three had active neuroborreliosis and one had an inflammatory arthropathy associated with active borreliosis.

If the presence of only bands 31 and 34 of IgG and/or IgM antibodies was considered a criterion for a positive WB, the number of positive patients would decrease to 11 (3.1 %), and three of the four patients with borreliosis would be detected (excluding the one that did not present band 34).

In group 2, 8 patients (16 %) were positive by EIA (Table 2), but none of these results could be confirmed by WB. In group 3, one patient had an absorbance value greater than 90 % of the cut-off value and a negative WB result (Table 2). All samples from patients in group 1 and group 3 were negative in the Rapid Plasma Reagin test and the test to detect fluorescent *Treponema* antibodies with absorbent. Samples from group 2 patients with syphilis were positive in both tests.

Diagnosis of Lyme disease in its early stages is difficult; most people are unaware of a tick bite, and the characteristic cutaneous manifestation, chronic erythema migrans, is absent in up to 50 % of patients. The majority of cases, therefore, characteristically present manifestations associated with more advanced stages of the disease, such as arthropathies (6) and predominantly inflammatory neuropathies. Given that Lyme disease may also occur with completely nonspecific manifestations, it is extremely difficult to diagnose. For this reason, clinical diagnosis should be confirmed by serological and CSF tests for IgG and IgM antibodies to *Borrelia burgdorferi*.

The prevalence of anti-*Borrelia burgdorferi* antibodies in our region is intermediate between values in the USA and those in the rest of Europe (7–9). One of the problems we must resolve is the choice of test to detect the presence of specific antibodies. Of the several methods currently available, immunofluorescent assay, EIA and WB are used most commonly. Most authors agree (10, 11) that EIA is the most suitable test for screening, given its high sensitivity and adequate specificity, and WB, with its greater sensitivity and specificity, is a suitable confirmatory method, reducing the number of false-positive results (11, 12). Our data confirm this, since the number of sera posi-

tive by the EIA test was reduced in all groups with the application of WB.

Another phenomenon described in the literature (13) and confirmed in this study is the limited value of studying intrathecal antibody production against *Borrelia burgdorferi* in subjects with symptoms compatible with neuroborreliosis, even though detection of an intrathecal antibody response may facilitate the correct diagnosis (14). In our study, intrathecal IgG antibodies were found only in one of the 117 CSF samples studied. The value of applying other diagnostic techniques to the CSF, such as the polymerase chain reaction, with or without nucleic acid hybridization, and antigen research using EIA has yet to be determined.

Positive WBs must be interpreted with great caution, given the large number of patients who may have false-positive results, such as those infected with herpes virus (15) or those suffering from lupus erythematosus. Cross-reactions with other bacteria either from the same (*Treponema pallidum*) (16) or a different family (*Bacillus subtilis*, *Salmonella typhimurium*) may also give rise to false-positive WB results (17). False-positive results are due mainly to homologies with a 41 kDa flagellar protein, which may be present also in other processes caused by these microorganisms. Thus, the detection of antibodies, even by WB, may indicate a previous contact with the microorganism, a present acute infection, a persistent chronic infection, an immunomediated postinfectious syndrome, a cross-reaction with a microorganism with which contact was made or a nonspecific clonal stimulation of B lymphocytes. In contrast to the findings of other studies (18), our patients in group 2 showed little cross-reactivity in the EIA and none in the WB.

The presence of antibodies to the flagellar protein of 41 kDa could cause many of the alterations that occur throughout the course of neuroborreliosis or that result from the presence of hidden antigens from the nervous system in inflammatory processes not due to *Borrelia burgdorferi*, since antigens similar to the 41 kDa protein have been detected in human tissue, especially in myelitic fibres of the central nervous system (19, 20). This could explain why antibodies to the 41 kDa protein were the most prevalent in our series. Alternatively, this higher prevalence could be due to the fact that the 41 kDa protein is one of the most immunogenic and nonspecific antigens of *Borrelia burgdorferi*.

In conclusion, the results of our study show that antibodies to *Borrelia burgdorferi* are not uncommon in our area, although actual Lyme disease is rare. When it occurs, Lyme disease mainly manifests as neuroborreliosis. Therefore, in our population it is advisable to consider Lyme disease in the differential diagnosis when presented with a patient with neurological disease of unknown etiology. In our region treatment for *Borrelia burgdorferi* infection should be administered when the patient presents symptoms characteristic of Lyme disease, after the possibility of other illnesses has been excluded and after the presence of IgG or IgM antibodies against *Borrelia burgdorferi* has been confirmed by a serological test that produces a minimum number of false-positive results.

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Comparison of Enzyme Immunoassay Antigen Detection, Nucleic Acid Hybridization and PCR Assay in the Diagnosis of *Chlamydia trachomatis* Infection

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An enzyme immunoassay (EIA) antigen detection system (MicroTrak, Syva), nucleic acid hybridization (PACE 2, Gen-Probe) and polymerase chain reaction (PCR) assay (Amplicor, Hoffmann-La Roche) were evaluated for the detection of *Chlamydia trachomatis* in a high-risk female population. Of 234 specimens, 42 (18 %) were positive. The respective sensitivity of the EIA, RNA hybridization and the PCR was 81, 90 and 88 %. When additionally performed on diluted specimens, PCR gave positive results for three of four PCR-negative specimens from EIA- and RNA-hybridization–positive women and a sensitivity of 95 %. Thus, both techniques employing gene technology offered a clear improvement in sensitivity over the EIA. Future improvements in the PCR should be directed towards the elimination of polymerase inhibition.

The enzyme immunoassay (EIA) antigen detection technique is the most widespread nonculture method in diagnostic laboratories handling large numbers of chlamydial specimens. The sensitivity of EIA antigen detection is generally considered satisfactory in high-risk populations such as patients with symptomatic acute chlamydial infections. In the detection of recently acquired chlamydial infections and in the treatment controls where the amount of infective particles may be low, however, the sensitivity of EIA antigen detection is clearly inadequate (1, 2). Moreover, problems due to the less than 100 % specificity of the EIA antigen detection methods (3, 4) are accentuated in low-risk populations where the frequency of false-positive test results may even outnumber that of true-positive test results. The

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