Western Blot as a confirmatory test for Lyme disease

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LYME DISEASE IS A MULTISYSTEM DISORDER WHICH MAY involve dermatological, musculoskeletal, nervous system or cardiac manifestations (1). The causative agent is a spirochete called Borrelia burgdorferi. Recent studies have shown that there is sufficient genomic and phenotypic heterogeneity among the strains commonly known as B burgdorferi to warrant their division into three genospecies: B burgdorferi, B garinii and a third, presently unnamed, genospecies (2).

Lyme disease has been reported from North America, Europe, Asia and Australia. Over 30,000 cases have been described from 46 American states, although most cases have been reported from only eight states (3). B burgdorferi has not been demonstrated in many states that have reported Lyme disease.

Lyme disease is less common in Canada. Only 140 cases were reported between 1984 and 1990 and, in fact, many of the reported cases would likely not meet the current Canadian surveillance case definition for Lyme disease (4). Field studies have shown the endemic presence of B burgdorferi at Long Point in southern Ontario (5) and B burgdorferi has been isolated from an Ixodes dammini tick collected in Prince Edward Island in 1991 (6). A closely related spirochete, B henselii, the etiological agent of tick-borne relapsing fever, has been found in the blood of patients in British Columbia (7,8). More recently, another spirochete (currently unidentified) has been observed in British Columbia ticks (personal communication).

The Laboratory Centre for Disease Control (LCDC) has co-sponsored a consensus conference on Lyme disease at which recommendations pertaining to epizootiology, epidemiology, clinical practice and laboratory investigation of Lyme disease were drafted (4). The Bureau of Communicable Disease Epidemiology, LCDC, maintains data on human Lyme disease cases occurring in Canada, and the Zoonotic Diseases section, National Laboratory for Special Pathogens of the Bureau of Microbiology, LCDC, provides laboratory support. This laboratory support includes collaborative investigations to determine the distribution of B burgdorferi in Canada, identification of ticks submitted, initiation of a joint venture to produce a manual on the identification and distribution of ticks in Canada, proficiency testing of provincial public health laboratories, provision of reference testing and evaluation of commercial products.

Three proficiency tests have been conducted from 1990-92 involving eight provincial public health laboratories. The serological tests reported included enzyme-linked immunosorbent assay (ELISA) performed by all laboratories as well as immunofluorescent antibody (IFA) and Western Blot (WB) tests undertaken by some laboratories. The results confirmed that ELISA is a more reliable screening assay than the IFA test, although false positive and negative serologies were obtained. Overall, ELISA sensitivities ranged from 42.9 to 100%, with most sensitivities over 90%, and specificities ranged from 75 to 100%. The few laboratories reporting WB results had sensitivities ranging from 50 to 100% and specificities from 60 to 100%.

It has been recommended that WB be used by Canadian laboratories to confirm the presence of specific antibodies in sera found positive by ELISA testing (4). The performance of the WB as a confirmatory test has been disappointing to date. Between August 1990 and December 1992, one or more ELISA positive sera from 40
patients were tested at LCDC using a commercial WB test with the following results: nine (22.5%) negatives, nine (22.5%) positives and 22 (55.0%) indeterminates. Thus, WB was able to provide a clear resolution of reactivity in only 45% of these patients.

There are no standard criteria for the interpretation of the WB test for Lyme disease. Some laboratories simply look for four or more designated bands whereas other laboratories require a specific pattern of reactive bands, eg, reaction with the 41 kDa flagellar protein band and at least one band corresponding to low molecular weight proteins of 18, 21.5 or 23 kDa (9). During infection there is an initial response to the 41 kDa protein followed over several months by a variable graded response to as many as 10 or more proteins. This delayed expression may be related to degradation of the structural integrity of the bacteria with resultant presentation of previously protected immunogenic constituents or perhaps due to increased suppressor cell activity, which has been demonstrated in early diseases may restrict the initial antibody response to the full spectrum of bacterial antigens (10).

Despite these inherent problems in the Lyme disease WB test, this test may still play a useful role. Rose et al (11) examined patients’ sera and showed that positive results from both WB and ELISA assays provided strong diagnostic support for Lyme disease whereas a positive ELISA with a negative WB was generally associated with a lack of clinical features of Lyme disease. Recently, Banerjee et al (12) used WB as a tool to demonstrate that IFA titres to B burgdorferi and B hermsii in British Columbia patients were likely due to nonspecific reactions and to conclude that it is unlikely borreliosis is a causative factor for any chronic arthropathies seen in British Columbia.

There are several commercial WB kits available and the Zoonotic Diseases section is planning to undertake an evaluation of these products in the coming year to determine their relative merits. It is important not to lose sight of the fact that diagnosis of Lyme disease requires appropriate clinical presentation with laboratory tests serving primarily as back up. Nevertheless, the recent isolation of B burgdorferi from the apparently nonendemic province of Prince Edward Island (6) illustrates the importance of having appropriate tests to recognize the sporadic cases that may occur in Canada.

REFERENCES