1060-0051/94/0401-0046
JOURNAL OF SPIROCHETAL AND TICK-BORNE DISEASES

Vol. 1, No. 2, 1994
Printed in U.S.A.

Does Lyme Borreliosis Exist in Australia?

B. J. Hudson,* M.D., R. D. Barry, B.VSc., D. R. Shafren, PhD., M. C. Wills, BSc., S. Caves, M.D., and V. A. Lennox

Microbiology Department, Royal North Shore Hospital, St. Leonards (B.J.H., V.A.L.) and Department of Microbiology, Newcastle University (R.D.B., D.R.S., M.C.W., S.C.)

The existence of an indigenous form of Lyme borreliosis (LB) in Australia has not yet been confirmed as isolation of the causative organism from clinical specimens collected from candidate patients has not yet been achieved. Candidate spirochetes, resembling *Borrelia burgdorferi* have been isolated from *Ixodes holocyclus* ticks but growth cannot be sustained in standard media. Erythema migrans, arthritis and radiculopathy have been described in candidate LB cases in Australia. Immunoblotting of sera from such cases indicates that antibodies to flagellin and OspA antigens of European isolates, *Borrelia garinii* and *Borrelia afzelii*, are commonly detected, while such serореactivity to *B. burgdorferi* sensu stricto is uncommon. Based on clinical and immunoblot data on candidate Australian LB cases, we postulate that an indigenous form of LB occurs in Australia, possibly caused by spirochetes more closely related to *B. garinii* and *B. afzelii* than *B. burgdorferi sensu stricto*.

Key words: Lyme, Indigenous, *Ixodes holocyclus*, Immunoblotting

LYME BORRELIOSIS IN AUSTRALIA

With few published clinical case reports of possible Lyme borreliosis acquired in Australia (1–3), the existence of a genuine, indigenous form of Lyme borreliosis (LB) in Australia has not yet been confirmed. Efforts to isolate *Borrelia (B.) burgdorferi* from candidate tick vectors have so far proved inconclusive (6). Despite this, we believe that there is an indigenous form of LB based on clinical and immunoblot data that we have collected since 1991. From the northern hemisphere experience, there is characteristically a delay between recognition of indigenous LB cases and isolation and identification of causative spirochetes. The Australian experience is likely to be similar. Furthermore, on an island continent with much unique native fauna, it would not be unexpected to find significant differences in LB epidemiology, clinical manifestations, and even etiological spirochetes when Australian and northern hemisphere LB are compared. Review articles always reference the first human case of indigenously acquired LB in Australia as a male who developed rash and subsequent arthritis following a bite from an "unidentified insect" in Hunter Valley, New South Wales (NSW) (1). While he appeared to have erythema migrans (EM), it is doubtful whether LB was the cause of arthritis. The next clinical case was reported from the NSW Central Coast, just north of Sydney in 1986 (2). The only other indigenous cases reported in refereed journals, both in the same letter, were from a coastal area south of Sydney in 1986 (3). All four indigenous cases had a rash consistent with EM.

None of the Ixodes species that transmit LB in the northern hemisphere are found in Australia, but another Ixodid tick, *Ixodes (I.) holocyclus*, commonly bites man, transmitting *Rickettsia australis*, the cause of spotted fever in Australia (Queensland tick typhus) and also causes a toxin-mediated paralysis in children and domestic dogs (4). Its distribution is along the eastern seaboard in Queensland, NSW, and Victoria, where it extends into the Great Dividing Range of mountains in some areas (5). Spirochetes that have both the morphology and some structural characteristics of Borreliae have been recovered from *I. holocyclus* but cannot currently be sustained in culture (see below) (6, 7).

In 1991, Wills et al. reported detection of spirochetes in cultures from engorged adult and nymphal *I. holocyclus* ticks (7). Isolation generally took 8 weeks, but contamination of cultures has remained a persistent problem, despite use of culture methods recommended by North American and European researchers (8, 9). Numbers of spirochetes from each culture are low and, when isolated, demonstrate extremely fastidious growth. Further study of the spirochetes is thus difficult. Despite this, 70/167 (42%) of ticks processed yielded spirochetes (7). The ticks were collected mostly in the Manning River district of NSW, a region in which we are now finding clinical cases (10). Purification and analysis of 4 isolates demonstrates that all have borrelia-like polyacrylamide gel electrophoresis (PAGE) profiles and react (albeit) weakly with a monoclonal antibody to OspA (H5332) (11). Polymerase chain reaction (PCR) products were obtained using primer sets for the flagellin (*fla*) and rRNA genes; one strain additionally produced a specific OspA product (Shafren et al., unpublished). Morphological resemblance to *B. burgdorferi* is shown by immunofluorescence with flagellin-specific monoclonal antibody (H9724) demonstrating spirochete morphology resembling that of *B. burgdorferi* (data obtained by Dr. V. Bundoc, University of Texas) (12). We therefore believe that there are Borreliae in Australian ticks that are structurally similar to the agents of LB as described in the northern hemisphere. Whether they are pathogenic for humans or animals requires correlation with clinical isolates when they are eventually made.

When investigating *likely* new endemic areas, persistently negative results from initial research cast doubts on whether local transmission of LB occurs. For example, Russell et al. obtained 78 spirochete isolates from 35 separate locations along the eastern seaboard of Australia (6). They processed over 10,000 ticks, comprising 12 species, but, following analysis of the spirochete isolates, they *queried* the existence of so-called *Lyme disease* in Australia (6). *Ixodes holocyclus* was shown in laboratory studies not to be a competent vector for B31 strain (13). These negative results have not deterred our research as one of us

*Corresponding author

(BJH) regularly sees clinical cases of LB acquired in Australia, examples of which are illustrated in Table 1. Failure to respond to standard treatment regimens has also not deterred our investigations, as every treatment regimen has a measurable failure rate, and currently, the optimal treatment for LB is not known (14). European researchers, in particular, have isolated spirochetes from skin and cerebrospinal fluid (CSF) in clinical relapses despite standard and even prolonged courses of intravenous antibiotics (15).

Despite increasing anecdotal reports of tick-bite-associated Lyme-like illness in eastern coastal Australia, and increasing referrals of sera to clinical diagnostic laboratories for Lyme serology, the only published body of data on which to base an Australian case definition are the three published cases of EM. Our initial case definition is based upon that developed by the United States Centers for Disease Control and Prevention (CDC), which, in itself, is problematic (16). It is acknowledged that this definition was developed for epidemiological purposes and, so, will exclude many clinical cases. Its use may also be inappropriate to another endemic area like Australia, especially since clinical manifestations of LB appear to vary with different genospecies of Borreliae from different parts of the world (17). If EM is either not common or not recognized, many LB cases will be missed. Definition of an endemic area before an isolate of the causative spirochete has been made in that area may have to be based on detection of cases with EM as in Steere's original investigation (18). We have seen a number of such cases and are confident that such clinical cases can define an endemic area in the initial stages of the investigation. The case definition can always be modified as knowledge improves. For serological confirmation of cases with manifestations other than EM, we chose the Western Blot method due to lack of specificity of screening tests like immunofluorescent antibody (IFA), enzyme-linked immunosorbent assay (ELISA), and hemagglutination tests (19). In immunoblots, we have tested for antibodies to the OspA (31kD) and flagellin (41kD) antigens. OspA appears to show little (if any) cross reactivity with organisms other than Borreliae that cause LB (20). OspA thus reprcsents the most likely unique antigenic protein of Lyme borreliae to which antibodies are made. Even though they are not very specific, showing considerable cross reactivity, antibodies to flagellin may be the only ones detected in early LB. As yet, we have not tested for IgM antibodies or for antibodies to low molecular weight proteins like the 21kD OspC, especially since OspC may not be expressed by some LB strains. Although the presence of antibodies to OspA may be specific to LB, early in the course of LB antibodies to OspA are not commonly seen; indeed, some persons never make antibodies to OspA (21). Accordingly, we acknowledge that sensitivity of immunoblots based upon the presence of antibodies to OspA will be low, but specificity was considered more important to assess whether indigenous cases of LB are occurring in Australia.

Table 1 displays clinical summaries for six patients seen by one of us (BJH) together with immunoblot data (Fig. 1–3). The patients live in known areas of tick infestation, along the eastern seaboard of NSW, although case 6 sustained the tick bite in Central Australia, outside the known area of distribution of *I. holocyclus*, the postulated tick vector of LB in Australia. Patients 5 and 6 had the typical EM rash, followed by systemic illness. Skin biopsies in cases 2, 5, and 6 showed lymphohistiocytic infiltrates, predom-

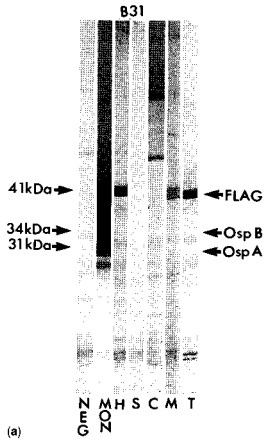
TABLE 1
Clinical Summaries and Immunoblot Data of Six Patients who Acquired Lyme Borreliosis in Australia (Adapted from Reference 12 with Permission)

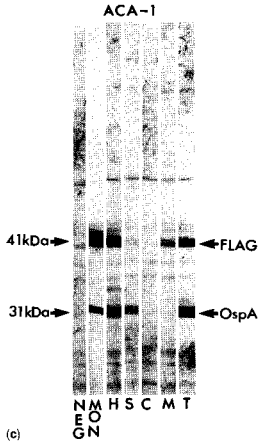
	Clinical Summary: Six Australian LB Cases[a]			
Case	Age/Sex	Illness (Duration)	Immunoblot	Therapy (Days)
1	71 female	Rash, radiculitis (4 months)	NBS16:F(+)A(+) ACA1:F(+)A(+) B31:F(+)A(−)	D(28)
2	47 male	Rash, CFS (12 years)	NBS16:F(+)A(+) ACA1:F(+)A(−) B31:F(+)A(+)	D(28) R ⊢ C (28)
3	9 male	Arthritis, fevers (4 months)	NBS16:F(+)A(−) ACA1:F(+)A(+) B31:F(+)A(−)	D(28) Cf(28) **H**
4	60 male	Rash, arthritis, fibromyalgia (3 years)	NBS16:F(+)A(−) ACA1:F(+)A(+) B31:F(+)A(−)	D(56) R + C (28) Cf(28) H
5	52 female	EM (recurrent) fibromyalgia (2 years)	NBS16:F(+)A(−) ACA1:F(+)A(−) B31:F(+)A(−)	D(28) Cf(14)
6	41 female	EM (recurrent) arthritis (14 months)	NBS16:F(+)A(+) ACA1:F(+)A(−) B31:F(−)A(−)	D(56) Cf(56) **H**

[a]Infections were all acquired in coastal NSW except case 6. EM = erythema migrans; CFS = chronic fatigue syndrome (by criteria of Holmes et al. (22); (fibromyalgia by criteria of Wolfe et al. (23)). Criteria for EM and arthritis followed CDC case definition (16).

ACA1, NBS16, B31 used for immunoblots: F = flagellin; A = OspA; (+) = positive; and (−) = negative.

Therapy: D = doxycycline 200 mg/day; R = roxithromycin 600 mg/day; C = cotrimoxazole 320/1600 mg daily; Cf = ceftriaxone 2 g/day; H = Herxheimer reaction with treatment. Days of treatment = total over 1 or more courses of therapy.





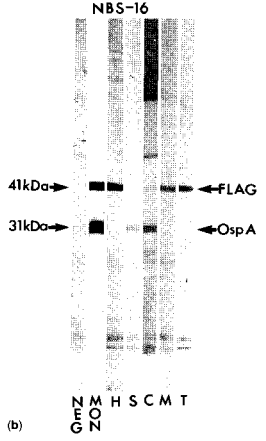


FIG. 1. Immunoblots on sera from Australian Lyme borreliosis cases using different species of B. burgdorferi *sensu lato*. *NEG = negative control; MON = monoclonal antibodies bound to 41kDa, OspB, and OspA proteins; H,C,M,T = cases 4, 2, 5, and 3, respectively, in Table 1. Immunoblots for cases 1 and 6 not shown (see text). (a) B31* (B. burgdorferi *sensu stricto*); *(b) NBS-16* (B. garinii); *(c) ACA-1* (B. afzelii).

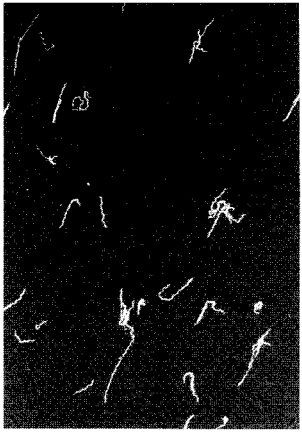


FIG. 2 B. burgdorferi *B31: immunofluorescence stain with flagellin monoclonal antibody H9724 (courtesy of Dr. V. Bundoc and Professor A. Barbour).*

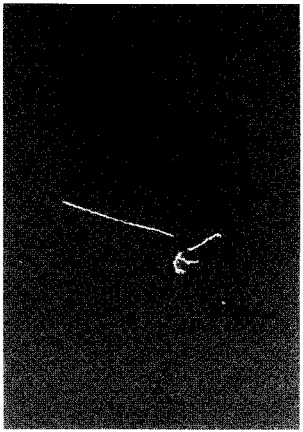


FIG. 3 Borrelia *isolated from engorged* I. holocyclus *ticks in eastern Australia: immunofluoresence stain with flagellin monoclonal antibody H9724 (courtesy of Dr. V. Bundoc and Professor A. Barbour).*

inantly perivascular and in dermis, except case 2 had significant infiltrate around a pilosebaceous follicle. Warthin-Starry silver stains for spirochetes were negative in all cases. Physician-observed joint swelling, for which no other cause was found, was required for the diagnosis of arthritis. Recurrent EM rashes were seen in cases 5 and 6 despite antibiotic treatment. Herxheimer reaction was not uncommon. Antibodies to OspA of North American strain B31 (*B. burgdorferi* sensu stricto) were not commonly detected, but antibodies to OspA of two European strains were. These belonged to the two other species groups of *B. burgdorferi* sensu lato: *B. garinii* (NBS-16) and *B. afzelii* (ACA-1), isolates from a tick in Sweden and an acrodermatitis chronica atrophicans skin lesion, respectively (provided by Professor A. G. Barbour, Texas).

We compared immunoblot results for patients judged likely to have LB based on clinical assessment by one of us (BJH) with healthy controls from the Newcastle area and for patients with connective tissue diseases. The clinical assessment was done independent of any knowledge of the immunoblot testing and vice versa. All patients had acquired their illness in Australia. For LB cases, 21/23 (>90%) had detectable Flagellin antibody by immunoblot while 13/23 (55%) had antibody to OspA. This contrasted with the low levels of antibodies to OspA seen in the other groups (Table 2). Differences were statistically significant.

We have found European strains of Borrelia most useful for immunoblotting in candidate Australian LB patients. Antibodies are detected to OspA of these strains in such patients rather than to OspA of the North American *B. burgdorferi* B31 strain (Table 3). When a local Australian clinical isolate is made, and can be grown in amounts adequate for immunoblotting, obviously we will study immunoblots with such isolates. Until then, northern hemisphere isolates will be used.

For individual cases, there was also variation in seroreactivity as the pattern of OspA positivity varied. Results were compared for 13 patients who produced antibodies to OspA. Of the 13 patients, 11 had antibody to NBS-16 OspA and 7 to ACA-1 OspA, but only 4 had detectable antibodies

TABLE 2
Immunoblot Reactivity LB Cases/Controls[a,b]

Category	Persons Tested	Number Positive	(%)
A. Healthy volunteers	92	2	(2.2)
B. Rheumatic illness—not LB	56	2	(3.3)
C. LB cases	23	13	(55.0)

[a]Seroreactivity to OspA proteins of European strains of *B. burgdorferi* of candidate Lyme borreliosis cases, compared with healthy controls and patients with connective tissue diseases. All patients had negative syphilis serology by TPHA (*Treponema pallidum* hemagglutination test) (adapted from Reference 12 with permission).

[b]European isolates NBS-16, ACA-1 used (see text)

A,B: not significant (Chi squared); A,C: significant $p < 0.01$; C,B: significant $p < 0.05$; and AB,C: significant $p < 0.01$.

TABLE 3
Immunoblot Reactivity of 686 Referred Sera[a,b]

Reactivity Pattern	Number	(%)
OspA + flagellin		
NBS-16 only	44	(6.4)
ACA-1 only	18	(2.6)
B31 only	4	(0.9)
NBS-16 & ACA-1 (both)	4	(0.6)
NBS-16 & B31 (both)	3	(0.4)
ACA-1 & B31 (both)	3	(0.4)
Subtotal	78	(11.3)
Trace OspA + flagellin	32	(4.6)
Strong flagellin (all strains)	50	(7.4)
Total	160	(23.3)

[a]Sera referred for Lyme serology 1/1/93–9/20/93.
[b]Three strains used: B31, NBS-16, ACA-1.

to OspA of both NBS-16 and ACA-1. This may indicate antigenic heterogeneity in causative spirochetes in Australia.

Because we have detected Borrelia-specific antibodies in the serum of candidate clinical cases of LB acquired in Australia, we hypothesise that an indigenous form of LB exists in Australia. The acquisition of at least one case outside the area of distribution of *I. holocyclus* indicates that ticks other than this species can transmit LB in Australia. The findings of Piesman and Stone should be reinterpreted in view of this observation (13). One could argue that the presence of antibodies to OspA, with or without antibodies to flagellin, is weak serological data for the existence of LB in Australia. Should not antibodies to antigens like OspC, the 94kD protein, and others also be sought? Should immunoblots use recombinant antigens (e.g., OspA protein or other immunogenic proteins)? We considered these approaches initially but discounted them because of increasing evidence of genetic diversity of spirochetes associated with LB worldwide. Heterogeneity of European isolates for OspA compared with North American isolates is well known. When comparing European to North American isolates, Barbour et al. demonstrated variable binding of monoclonal antibodies to OspA proteins as well as different arrangements of OspA-associated DNA sequences (24). In a newly described endemic area in Japan, four of eight clinical *B. burgdorferi* sensu lato isolates failed to react with H5332 but still possessed OspA-like protein bands on SDS-PAGE analysis, indicating different epitopes compared with European and North American strains (25). Using restriction fragment length polymorphism (RFLP) analysis, virtually all clinical isolates in Japan were dissimilar to representative isolates from Europe and North America (25).

We feel that our approach is also supported by the work of Wilske et al. (26) who, using a panel of monoclonal antibodies for OspA, identified at least seven different OspA serotypes with one other OspA serotype that did not bind any of the monoclonal antibodies. Of 128 OspA-expressing strains, serotype 1 corresponded to *B. burgdorferi* sensu stricto, serotype 2 to *B. afzelii*, and serotypes 3 through 7 to *B. garinii*; serotype X was the label given to three strains from *I. dentatus* ticks that had varying molecular mass of OspA proteins and varying binding patterns for monoclonal antibodies. Only one monoclonal antibody bound to all seven OspA scrotypes. Analysis of OspA partial amino acid sequences showed highly conserved regions but a significant variable region. To detect local cases of LB in Australia, recombinant OspA and other proteins for immunoblots may lack essential epitopes and may well be inappropriate. The demonstrated geographic genetic heterogeneity of spirochetes and OspA serotypes is likely to be of relevance to LB in Australia and to the identity of putative local spirochetes. Since *B. burgdorferi* has been isolated from migratory birds and their ticks (27, 28), and migratory birds travel annually from LB endemic areas in northern Japan and eastern Asia to the whole Australian east coast, one would suspect that some (possibly most) Australian isolates are likely to more resemble isolates from these areas than areas in North America or Europe. Recombinant proteins from well-characterized North American strains may be of little use for immunoblotting in Australian LB cases.

More appropriate is our approach to perform immunoblots using strains from the 3 different genospecies of *B. burgdorferi* sensu lato. We also feel that recent work by Assous et al. further supports this approach (29). They performed immunoblots on sera from known LB patients with EM, meningoradiculitis, arthritis or acrodermatitis chronica atrophicans (ACA) using strains from the three different species groups of *B. burgdorferi* sensu lato. For patients with EM or meningoradiculitis, against *B. afzelii* (two strains), no antibodies to OspC were detected, and in few cases were antibodies to the 94kD protein detected; against *B. garinii* (three strains), antibodies were detected to OspC in most cases but not to 94kD in every case; against *B. burgdorferi* sensu stricto (three strains), no antibodies were detected to OspC in many cases. For the same strains with sera from patients with ACA or arthritis, antibodies to OspC were only variably detected. We feel that these serological reactions to different genospecies resemble those we report for OspA and flagellin. Lack of detection of antibodies to OspC and the 94kD protein may be relevant to Australian patients, a point that we are currently addressing.

The clinical manifestations of LB in Australia appear to resemble those seen in the northern hemisphere. The spectrum of illness and frequency of clinical manifestations remain to be determined however. For the moment, treatment recommendations follow northern hemisphere guidelines recognizing that optimal therapy is yet to be established (14). Treatment of chronic cases of LB is problematic as persistent symptoms and relapses are seen often in the northern hemisphere (14). Similar problems have been observed in Australian cases, as illustrated by 4 of the 6 cases presented in Table 1, where repeat courses of oral and intravenous antibiotics have been given. This need was particularly obvious in cases 5 and 6 where recurrent EM occurred. Case 1 has just commenced ceftriaxone therapy. The only case of complete resolution of symptoms and signs and apparent cure was case 3, following ceftriaxone therapy. Incorrect diagnosis and/or persisting coexistent illness to explain poor response to therapy is possible, but physician-observed recurrences of the original EM rash in cases 5 and 6 are best explained by failure of antibiotic therapy.

We gratefully acknowledge the help and encouragement of Professor A. G. Barbour, Drs. V. Bundoc, and D. Thomas of University of Texas Health Sciences Center at San Antonio who kindly provided a variety of *B. burgdorferi* strains and the monoclonal antibodies H5332, H6831, and H9724.

Generous financial assistance has been provided by Dr. S. Buckingham and Mr. A. Sceney, Roche Products Australia, and by the Arthritis Foundation of Australia.

Reprint requests: B. J. Hudson, M.D., Microbiology Department, Royal North Shore Hospital, St. Leonards.

REFERENCES

1. Stewart A, Glass J, Patel A, et al. Lyme arthritis in the Hunter Valley. Med J Aust 1:139, 1982.

2. Lawrence RH, Bradbury R, Cullen JS. Lyme disease on the NSW central coast (letter). Med J Aust 145:364, 1986.
3. McCrossin I. Lyme disease on the NSW south coast (letter). Med J Aust 144:724–725, 1986.
4. Sutherland SK. *Australian Animal Toxins*. Oxford, Oxford University Press, 1983, pp. 299–315.
5. Seddon HR. Disease of domestic animals in Australia. Part 3. Tick and mite infestations. Service publication no. 7. Commonwealth of Australia, Department of Health, Canberra, 1951.
6. Russell RC, Munro R, Doggett SL, et al. Does Lyme borreliosis occur in Australia? Programs and Abstracts V Int Conf on Lyme borreliosis, Arlington, Virginia, USA, May 30–June 2, 1992, Abstract No. 332.
7. Wills MC, Barry RD. Detecting the cause of Lyme Disease in Australia. Med. J. Aust. 155:275, 1991.
8. Barbour AG. Laboratory aspects of Lyme borreliosis. Clin Microbiol Rev 1:1711–1719, 1988.
9. Preac-Mursic V, Wilske B, Schierz G. European *Borrelia burgdorferi* isolated from humans and ticks. Culture conditions and antibiotic susceptibility. Zentralb Bakteriol Mikrobiol Hyg A 263:112–118, 1986.
10. Barry RD, Shafren DR, Wills MC, et al. B.J. Lyme disease in Australia. Proc 2nd Eur Symp Lyme Borreliosis, London, 407, 1993.
11. Shafren DR, Wills MC, Barry RD. Antigenic properties of *Borrelia burgdorferi* isolated from *Ixodes holocyclus* and other ticks in eastern Australia. Programs and Abstracts V Int Conf on Lyme borreliosis, Arlington, Virginia, USA, May 30–June 2, 1992, Abstract No. 256.
12. Hudson BJ, Barry RD, Shafren DR, et al. Evidence for Lyme Disease in Australia. Today's Life Sciences. Thomson Business Publishing, Chippendale, Sydney, Australia, 1994 (in press).
13. Piesman J, Stone BF. Vector competence of the Australian paralysis tick *Ixodes holocyclus* for the Lyme disease spirochete *Borrelia burgdorferi*. Int J Parasitol 21(1):109–111, 1991.
14. Liegner KB. Lyme disease: the sensible pursuit of answers. J Clin Microbiol 31(8):1961–1963, 1993.
15. Preac-Mursic V, Weber K, Pfister HW, et al. Survival of *Borrelia burgdorferi* in antibiotically treated patients with Lyme borreliosis. Infection 17(6):355–359, 1989.
16. Centers for Disease Control. Case definition for public health surveillance. MMWR, 39(RR-13):19–21, 1990.
17. van Dam AP, Kuiper H, Vos K, et al. Different genospecies of *Borrelia burgdorferi* are associated with distinct clinical manifestations of Lyme borreliosis. Clin Inf Dis, 17:708–717, 1993.
18. Steere AC, Malawista SE, Harden JA, Ruddy S, Askanase PW, Andiman WA. Erythema chronicum migrans and Lyme arthritis. The emerging clinical spectrum. Ann Intern Med 86:685–698, 1977.
19. Duffy J, Mertz LE, Wobig GH, Katzman JH. Diagnosing Lyme disease: the contribution of serological testing. Mayo Clin Proc 63:1116–1121, 1988.
20. Bruckbauer HR, Preac-Mursic V, Fuchs R, Wilske B. Cross-reactive proteins of Borrelia burgdorferi. Eur J Clin Microbiol Infect Dis 11:224–232, 1992.
21. Zoller L, Cremer J, Faulde M. Western blot as a tool in the diagnosis of Lyme borreliosis. Electrophoresis 14:937–944, 1993.
22. Holmes G.P., Kaplan J.E., Gantz N.M. Chronic fatigue syndrome: a working case definition. Ann Intern Med 108:386–389, 1988.
23. Wolfe F, Smythe HA, Yunus MB, et al. The American College of Rheumatology 1990 criteria for the classification of fibromyalgia. Report of the multicenter criteria committee. Arth Rheum 33:160–172, 1990.
24. Barbour AG, Heiland RA, Howe TR. Heterogeneity of major proteins in Lyme disease borreliae; a molecular analysis of North American and European isolates. J Infect Dis 152(3):478–484, 1985.
25. Fukunaga M, Sohnaka M, Nakao M, et al. Evaluation of genetic divergence of borrelial isolates from Lyme disease patients in Hokkaido Japan by rRNA gene probes. J Clin Microbiol 31(8):2044–2048, 1993.
26. Wilske B, Preac-Mursic V, Gobel UB, et al. An OspA serotyping system for *Borrelia burgdorferi* based on reactivity with monoclonal antibodies and *ospA* sequence analysis. J Clin Microbiol 31(2):340–350, 1993.
27. McLean RG, Ubico SR, Norton Hughes CA. et al. Isolation and characterization of *Borrelia burgdorferi* from blood of a bird captured in the Saint Croix River Valley. J Clin Microbiol 31(8):2038–2043, 1993.
28. Olsen B, Jaenson TGT, Noppa L, et al. A Lyme borreliosis cycle in seabirds and *Ixodes uriae* ticks. Nature 362:340–341, 1993.
29. Assous MV, Postic D, Paul G, et al. Western blot analysis of sera from Lyme borreliosis patients according to the genomic species of the Borrelia strains used as antigens. Eur J Microbiol Infect Dis 12(4):261–268, 1993.