Citation: Banerjee, S.N., Banerjee, M., Smith, J.A., and Fernando, K. 1994. Lyme Disease in British Columbia—an update. *In*: Proceedings of the VII Annual Lyme Disease Foundation International Conference, Stamford, Connecticut, 22-23 April 1994, 88 p.

LYME DISEASE IN BRITISH COLUMBIA - AN UPDATE

S. N. Banerjee, Ph.D.[1,2], M. Banerjee, Ph.D.[2],
J. A. Smith, M.D.[1,2] and K. Fernando, M.Sc.[1]

[1] Provincial Laboratory, B.C. Centre for Disease Control
[2] Department of Pathology and Laboratory Medicine,
University of British Columbia, Vancouver, B.C.

Abstract: The results of 16,286 specimens submitted for Lyme disease test to the Provincial Laboratory during April 1986 to December 1993 have been reviewed and analysed. A total of 25 positive cases for Lyme disease were confirmed. Epidemiological data suggest that 7 of these patients were infected in British Columbia. Borrelia spirochetes were isolated from tick vectors collected by flagging and retrieval from trapped rodents from 20 different areas in B.C. Six motile and 14 non-motile spirochete cultures were isolated from juvenile *Ixodes angustus* and adult *Ixodes pacificus* ticks from 10 different areas. Eleven non-motile cultures were isolated from mice trapped in 4 different areas of B.C. All isolates were immunostained positive with monoclonal antibodies for OSPA, OSPB, P39 and flagellin of *B. burgdorferi*, the causative agent for Lyme disease and were positive by polymerase chain reaction for OSPA. The discovery of Lyme spirochetes in *I. angustus* larvae and *I. pacificus* ticks suggest a 2 tick cycle in B.C. This information is important to clinicians who are called upon to deal with patients who have been bitten by ticks. The Ministry of Health has produced a pamphlet on this subject titled "Tick Bites" which is recommended to all physicians.

Erythema migrans (EM) rash, a characteristic skin lesion for Lyme disease was recognized in 1975[1,2]. The causative agent was isolated in 1982 by Dr. William Burgdorfer from *Ixodes dammini* (now called *I. scapularis*) ticks[3]. In Canada, Long Point and Point Pelee in Ontario are the only areas where spirochete infected *I. scapularis* ticks and wild rodents have been found[4]. In British Columbia, there has been a dramatic increase in the number of specimens submitted for Lyme disease to the Provincial Laboratory since 1986[5]. Most of the patients had symptoms compatible for Lyme Borreliosis; however, very few of them had EM type of rash.

During the period of April 1986 to December 1993, our laboratory analysed over 16,000 specimens sent for tests for Lyme disease. Although there was over 20% incidence of reactivity on IFA testing for *Borrelia burgdorferi* ($\geq$1:256), results of EIA and Western Blots confirmed only 25 positive cases for Lyme disease. Epidemiological data of the positive patients revealed 18 of these patients had travelled outside B.C. to areas described as endemic for Lyme disease and, therefore, may have acquired the infection outside the province. The other seven cases were found in different areas in B.C. and none of these patients exhibited the typical EM rash following tick bites (Table 1). This necessitated a thorough investigation of ticks, wild rodents and human populations in B.C. for the isolation of the etiologic agent. Seroprevalence studies for Lyme-like disease in human and wild life were also necessary.

In a seroprevalence survey of Borreliosis in children with chronic arthritis in B.C., no significant differences amongst arthritic patients and controls for the distribution of IFA titers were seen. All the high titered sera when further examined by Western Blot tests were confirmed as non-reactive[6].

At the beginning of 1993 we selected 20 sites in different parts of B.C. on the basis of localized tick infestation or proximity of patients with Lyme disease. Adult and juvenile ticks were collected by flagging over vegetation and by retrieving ticks from trapped animals. Ticks were surface sterilized by 10% hydrogen peroxide and 70% isopropanol prior to dissection. Only gut tissues were cultured in BSKII medium containing kanamycin (4 ug/ml), nalidixic acid (50 ug/ml), 5-fluorouracil (100 ug/ml) and rifampin (25 ug/ml) either individually or in pool. Rodents were sacrificed by euthanasia and 6 organs, viz ear, bladder, kidney, spleen, liver and heart were cultured in BSKII medium for 202 rodents.

All cultures were examined by dark field microscopy every week up to 20 weeks. Any culture showing spirochetes was subcultured in BSKII medium without antibiotics. Smears of cultures were stained by Giemsa and immunostained with the following monoclonal antibodies: H5332, H9724 and H6831 and H11F3. All isolates from an area where spirochetes were seen were further tested by polymerase chain reaction using primary tick cultures and nested primers for OSPA gene and by monoclonal antibody tests for *B. burgderferi* (Table 2, 3). Fourteen isolates were evaluated using anti-mouse monoclonal antibodies against 4 specific Lyme antigens (Table 4). All isolates were further evaluated serologically by indirect immunofluorescence (IFA) with patients' sera and compared with similar tests using *B. burgdorferi* - B_{31} strain. Results of IFA test on one isolate (#382) is shown in Table 5. Electron microscopy of contaminated cultures revealed spiral structures similar to Lyme disease spirochetes. Four of the motile cultures were subjected to DNA sequence for 16SrRNA gene analysis using universal primers (Banerjee and Altamirano, unpublished).

During the period of February to December 1993, we have cultured 2,500 ticks collected from 20 selected sites. Tick species collected were as follows: *I. pacificus, I. angustus, I. soricis, Dermacentor andersoni and D. albipictus.* At the same time we have cultured six tissues from each of 202 mice trapped in those areas.

We have seen spirochetes in 20 tick cultures and 11 organ cultures from mice collected at 10 different sites in B.C. We isolated motile spirochetes in 6 separate tick cultures from 5 different sites in B.C geographically far apart. Three of these motile spirochetes were isolated from adult *I. pacificus* ticks and three were isolated from pools of 4/5 larvae of *I. angustus* ticks. Mice from which these larvae were removed also revealed the presence of spirochetes in different organs, the most common being ear lobe, kidney and heart. However, some of the mouse isolates were non-motile suggesting the presence of dead organisms. It appears that our medium may be deficient in some important ingredients for supporting the growth and survival of B.C. isolates.

All our isolates were from *I. pacificus* adults and *I. angustus* juvenile ticks. One *I. pacificus* larva on a lizard also revealed the presence of spirochetes. However, the lizard being a protected species was allowed to escape. It appears that the tick vectors *I. pacificus* and *I. angustus* and hosts like deer mouse (*Peromyscus maniculatus*) and Northern alligator lizard (*Gerrhonotus ceruleus principis*), are involved in transmitting Lyme-like disease in B.C.

All spirochete cultures were positive by PCR in both nested and unnested primer experiments using two different sets of primers for OSPA gene. Spirochetes were also immunostained by the antimouse monoclonal antibody for flagellin, OSPA, OSPB and P39 antigens (courtesy of Dr. T. Schwann, Rocky Mountain Labs., MT, U.S.A. and Dr. J. F. Anderson, Connecticut Agricultural Experimental Station, New Haven, CT, U.S.A.)[7,8,9]. A comparison of IFA results using *B. burgdorferi* and B.C. isolates using a panel of patients' sera showed that these spirochetes are antigenically related to the etiologic agents of Lyme disease (Table 5). The monoclonal antibody tests confirm that the new spirochete isolates share at least 4 antigens of *B. burgdorferi* and therefore can be identified as *B. burgdorferi* (Table 4). DNA sequence for 16SrRNA gene was determined for the 4 axenic cultures. These sequences were compared with stored sequence in the Gen Bank. There were good matchings between the nucleotide analysis of the cultures and those of *B. burgdorferi* sequences stored in the Gen Bank.

The discovery of spirochetes in 3 cultures from pools of *I. angustus* larvae from different areas in B.C. is the first such finding involving *I. angustus* ticks with Lyme disease. In the neighbouring state of Washington there was a case report by Damrow et al [10]. A 3-year old child bitten by *I. angustus* tick developed an EM rash and positive Lyme serology. The presence of spirochetes in both *I. pacificus* and *I. angustus* ticks suggest a 2-tick cycle for human infection in British Columbia[11]. Further research is in progress to determine whether the symptomatology seen in B.C. patients are connected with our isolates.

Isolation of spirochetes in axenic form has been done for 4 isolates in the B.C. Provincial Laboratory. Similar attempts are being made for the rest of our isolates for future works on antigenic and genetic characterization of the Borrelia species isolated in British Columbia.

References

1. Steere, A.C., Malawista, A.C., Snydam, D.R. et al: Lyme arthritis: an epidemic of oligomticular arthritis in children and adults in three Connecticut communities. Arthritis Rheum 1977;20:7-17.

2. Steere, A.C., Gibofsky, A, Patarroyo, M.E. et al: Chronic Lyme arthritis: clinical and immunogenetic differentiation from rheumatoid arthritis. Ann Intern Med 1979;90:896-901.

3. Burgdorfer, W., Barbour, A.G., Hayes, S.F. et al. Lyme Disease - a tick-borne spirochetosis? Science 1982;216:1317-1319.

4. Consensus Conference on Lyme Disease; Can J Infect Dis 1991;2:49.

5. Banerjee, S.N. and Smith J. A. A review of Borreliosis in B.C., April 1987 to March 1989. Dis Surv 1989;9:199-207.

6. Banerjee, S.N., Banerjee, M., Cimolai, N. et al. Seroprevalence survey in Borreliosis in children with chronic Arthritis in British Columbia, Canada. J Rheumatol 1992;19:1620-1624.

7. Barbour, A.G., Tessler, S.L. and Todd, W.J. Lyme disease spirochete and ixodid tick spirochetes share a common surface antigenic determinant defined by a monoclonal antibody. 1983; Infect Immun 41:795-804.

8. Barbour, A., Tessier, Hayes, S.F. Variation in a major surface protein of Lyme disease spirochetes. 1984; Infect Immun 45:94-100.

9. Schwan, T., Schrumpf, M.E., Karstens, R.H. et al. Distribution and molecular analysis of Lyme disease spirochetes *Borrelia burgdorferi* isolated from ticks throughout California. 1993; J Clin Microbiol 31:3096-3108.

10. Damrow, T., Freedman, H., Lane, R.S. and Preston, K. Is *Ixodes (Ixodiopsis) angustus* a vector of Lyme disease in Washington State? 1989; West J Med 150:580-582.

11. Banerjee, S.N. Isolation of *Borrelia burgdorferi* in British Columbia. 1993; B.C. Health & Dis Surv 10:92-93.

Acknowledgement

Funding for this ongoing study is provided by the Ministry of Health and Ministry Responsible for Seniors, Province of British Columbia and a grant from the Vancouver B.C. Lyme Borreliosis society. We thank Dr. S. Byrne at Provincial Laboratory, B.C. Centre for Disease Control for help in establishing PCR methods for Borreliosis in our laboratory. We thank Dr. J. F. Anderson, Director, Connecticut Agricultural Experimental Station for helping us in the identification of the isolates.

Table 1

CONFIRMED LYME DISEASE CASES IN BRITISH COLUMBIA
April 1986 to December 1993

Total confirmed Lyme disease cases in B.C. : 25

Lyme disease cases acquired in B.C. : 7

PT	AGE	SEX	LOCATION	YEAR	SYMPTOMS
O.B	2	F	Burns Lake	1988	Fever, headache, rash, fatigue.
A.L.	44	F	Salt Spring Island	1989	Rash, cellulitis, headache, muscle pain, rigor.
S.J	71	F	Galiano Island	1989	Rash - erythema multiforme, arthritic pain headache, joint effusion.
C.R	46	M	Kootenay Lake District	1992	Fever, fatigue, rigors, parasthesia, arthralgia, myalgia.
J.H.	66	M	Cories Island	1993	Arthralgia, joint swelling, joint effusions, no EM rash.
B.B.	58	F	Oliver	1993	Rash, facial palsy, tingling sensation in finger tips, extending to all fingers. Started as a boil on arm prior to brachial plexopathy.
W.P	74	F	Nanaimo	1993	Rash - erythema migrans, fever, fatigue, arthralgia, myalgia. Treated with Doxycyline - Got better.

Table 2

Isolation of spirochetes from ticks and wild mice in B.C.
and tested by Polymerase chain reaction or monoclonal antibody tests for OSPA gene

Culture #	Tick species	Place in B.C.	Darkfield microscopy of spirochetes	PCR/ Mab results	Comments
115	5♂ *I. pacificus*	Bowen Island	Non-motile	Pos.	
202	2♂ *I. pacificus*	Harrison	Non-motile	Pos.	
334	2 larvae *I. pacificus*	Metchosin	Non-motile	Pos.	On mouse
344	1 larva *I. pacificus*	Metchosin	Non-motile	Pos.	On lizard
363	2♂ *I. pacificus*	Metchosin	Non-motile	Pos.	
368	3♀ *I. pacificus*	Metchosin	Non-motile	Pos.	
382	3♂ *I. pacificus*	Metchosin	Motile	Pos.	
664	3 larvae *I. pacificus*	Cultus Lake	Non-motile	Pos.	
665	5 larvae *I. angustus*	Cultus Lake	Motile	Pos.	On mouse
703	2♀ *I. pacificus*	Cultus Lake	Motile	Pos.	
710	2♀ *I. pacificus*	Cultus Lake	Non-motile	Pos.	
728	2♀ *I. pacificus*	Nanoose Bay	Non-motile	Pos.	
936	2♂ *I. pacificus*	Hope	Motile	Pos.	
1340	4 larvae *I. angustus*	Squamish	Motile	Pos.	On mouse
1545	4 Larvae *I. angustus*	Galiano Island	Motile	Pos.	On mouse
1778	1 *I. angustus* larva	Sechelt	Non-motile	Pos.	On mouse
1779	2 *I. angustus* larvae	Sechelt	Non-motile	Pos.	On mouse
72	10 *I. pacificus* ♀	Lasqueti Isl.	Non-motile	Pos.	On goat
1990	5 *I. pacificus* ♀	Lasqueti Isl.	Non-motile	Pos	On cat
1992	2 *I. pacificus* ♀	Lasqueti Isl.	Non-motile	Pos	On cat

Table 3

Isolation of spirochetes from different organs of wild mice in B.C. and tested by PCR or monoclonal antibody tests

Culture #	Tick species/ mouse	Place in B.C.	Darkfield microscopy of spirochetes	PCR/ Mab results	Comments
W61	*P. maniculatus*	Harrison	Non-motile	Pos.	Heart
W62	*P. maniculatus*	Metchosin	Non-motile	Pos.	Ear, heart
W66	*P. maniculatus*	Metchosin	Non-motile	Pos.	Kidney
W69	*P. maniculatus*	Metchosin	Non-motile	Pos.	Kidney, spleen
W70	*P. maniculatus*	Metchosin	Non-motile	Pos.	Ear, kidney, spleen
W73	*P. maniculatus*	Cultus Lake	Non-motile	Pos.	Liver, heart
W77	*P. maniculatus*	Cultus Lake	Non-motile	Pos.	Ear, kidney, spleen, liver, heart
W183	*P. maniculatus*	Lasqueti Isl.	Non-motile	Pos.	Ear, bladder, kidney, spleen
W185	*P. maniculatus*	Lasqueti Isl.	Non-motile	Pos.	Ear, bladder, heart
W189	*P. maniculatus*	Lasqueti Isl.	Non-motile	Pos.	Bladder, kidney, heart
W190	*P. maniculatus*	Lasqueti Isl.	Non-motile	Pos.	Ear, bladder

Table 4

Serologic evaluation of spirochete isolates using monoclonal antibodies

Culture #	H5332 (31KD)	H9724 (41KD)	H4610 H6831 (34KD)	H11F3 (39KD)
B31	Pos.	Pos.	Pos.	Pos.
382	Pos.	Pos.	Pos.	Pos.
115	Pos.	Pos.	Pos.	Pos.
202	Pos.	Pos.	Pos.	Pos.
665	Pos.	Pos.	Pos.	Pos.
728	Pos.	Pos.	Pos.	Pos.
W77	Pos.	Pos.	Pos.	Pos.
703	Pos.	Pos.	Pos.	Pos.
936	Pos.	Pos.	Pos.	Pos.
1340	Pos.	Pos.	Pos.	Pos.
1545	Pos.	Pos.	Pos.	Pos.
1778	Pos.	Pos.	Pos.	Pos.
1779	Pos.	Pos.	Pos.	Pos.
W72A	Pos.	Pos.	Pos.	Pos.

Table 5

Serologic evaluation of patients' sera on Lyme disease spirochetes and B.C. isolate #382

Patient No.	Status	IFA titres using *B. burgdorferi*	IFA titres using 93T382	Comments
1247	Pos. for Lyme	1:16384	1:16384	Reactive
879	Pos. for Lyme	1:1024	1:1024	Reactive
912	Pos. for Lyme	1:512	1:1024	Reactive
983	False Pos. for Lyme	1:512	1:256	Reactive
1035	False Pos. for Lyme	1:512	1:512	Reactive
1008	Neg. for Lyme	1:128	1:64	Non-reactive
1036	Neg. for Lyme	1:64	1:64	Non-reactive

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W P	74	F	Nanaimo	1993	Rash - erythema migrans, fever, fatigue, arthralgia, myalgia. Treated with Doxycyline - Got better.

Addendum

Collection Year	Culture	Location	GenBank No.
1993	BC93T1340	Squamish	AY077830

This isolate was later delineated as *Borrelia bissettiae* (formerly *Borrelia bissettii*), which is pathogenic to humans.

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