Seroprevalence of *Borrelia burgdorferi* in North Eastern India

Surg Cmde AK Praharaj*, Lt Col S Jetley (Retd)[†], Col AT Kalghatgi[#]

Abstract

Background: There is paucity of data on Lyme disease in India. A seroprevalence study of *B burgdorferi* infection was carried out in North-Eastern states of India to assess the same.

Methods: Sera from 500 individuals of North-Eastern states of India were tested for IgG antibody by enzyme linked immunosorbent assay using commercial kits containing recombinant antigen.

Result: Out of 500 persons, 65 (13%) were positive for *B burgdorferi* specific IgG. Females showed higher positivity rate as compared to males (15.86% vs 10.95%). Higher prevalence rate was observed in the age group of 15-30 years in both sexes (11.48% in male and 18.69% in female). Arunachal Pradesh showed higher seroprevalence rate (17.8%) as compared to other North-Eastern states (8.46-9.6%).

Conclusion: Seropositivity to *B burgdorferi* suggests infection by the organism and presence of Lyme disease in these areas. Further population and vector biology studies are required to find out the exact species involved in transmission of the organism.

MJAFI 2008; 64 : 26-28

Key Words: Lyme Disease; Seroprevalence; *Borrelia burgdorferi*

Introduction

Lyme disease was recognised in 1975 in Lyme county of Connecticut, United States in children suffering from arthritis. The illness was due to a spirochaete *Borrelia burgdorferi* that was isolated from the skin lesion called erythema chronicum migrans (ECM) [1]. The disease was transmitted by the bite of the ticks of *Ixodes ricinus* complex mainly *I dammini*. Deer have been found to be the reservoir of the organism [2]. The disease has a protean manifestation affecting many systems that include skin, joints, muscle, central nervous system, heart and eye [3]. This infection produces both IgG and IgM response in the host to various proteins of the organism. These proteins include OspC (22 KD OspC, p22), 41kD flagellar antigen, 58kD heat shock protein, 31kD OspA and 38KD OspB. The early serological response is mostly directed against 22 kD OspC and p22 of the spirochete. After four to six weeks of infection most patients seroconvert and IgG antibody persists for long duration. The initial response is of the IgM isotype followed by IgG class. About 5-10% of cases are asymptomatic and only low levels of IgG antibodies can be detected by enzyme linked immunosorbent assay (ELISA) [4]. Although the organism can be cultured from the skin lesion and joint fluid, the isolation rate varies from 2-33% in various

studies. Highest isolation rate has been reported from skin lesion [5].

The disease is endemic in United States with its seroprevalence ranging from 2-12% [6]. It has been reported from Europe, Middle-East, South-East Asia, former Soviet Union and Australia [7, 8]. The incidence in India is not known and this study was under taken to study the seroprevalence of *Borrelia burgdorferi* infection in north eastern parts of India.

Material and Methods

The study population included service personnel and their families from north eastern region of India comprising Arunachal Pradesh, Meghalaya, Manipur, Nagaland and Assam. A total of 500 subjects of different age and sex group were included. Five millilitre of venous blood was collected from each individual in a sterile bottle, serum separated and frozen at -20°C till further use.

IgG antibody to *Borrelia burgdorferi* was detected by commercial ELISA kit (NovaTech, Germany). The kits are coated with recombinant antigens of *Borrelia burgdorferi* flagellar antigen (41kDa, p41), OspC of the phylum B₃₁ (*B sensu stricto*) 20047 and T25 (*B garinnyi*) OspC, p100 and p18 of the phylum PKO=Afzillii and p41i garinnyi (PB1). The species specific marker p100 and p18 are especially reliable for detecting the IgG response. p41i is included in the presented antigens to avoid cross reactivity with antibodies of syphilitic sera.

*PDMS(HS), DGMS Navy, Naval HQ, Delhi. [†]Ex-Classified Specialist (Pathology), AFTC, Delhi Cantt. [#]Director (Pension), Office of DGAFFMS, Ministry of Defence, M Block New Delhi.

Ten known VDRL positive sera were tested to check the kit for false positivity. Sera positive for IgG antibody to *Borrelia burgdorferi* were also tested for VDRL, *T pallidum* hemagglutination test (TPHA), antinuclear antibody (ANA), rheumatoid factor (RF) and Weil-Felix reaction to find out false IgG positivity to *Borrelia burgdorferi*. The test procedure was carried out as per manufacturer's instructions. All the samples were tested in duplicate along with positive and negative controls.

Results

All 10 VDRL positive sera tested, were found negative for IgG antibody to *Borrelia burgdorferi*. Age and sex-wise distribution of IgG positivity is given in Table 1. A total of 32 (10.95%) male subjects and 33 (15.86%) females were positive for IgG antibody to *Borrelia burgdorferi*. Overall positivity in both sexes was 13%. Arunachal Pradesh had highest (17.8%) seroprevalence rate (Table 2).

All the positive samples were tested for VDRL, TPHA, ANA, RF and Weil-Felix reaction. One sample was found positive for both VDRL and TPHA, and one positive for ANA. Data of both these cases has been excluded.

Discussion

The Ixodes ticks are present in Himalayan region of India [9] and therefore there is a possibility that lyme disease may exist in our country. Patial et al [10], reported a case of Lyme disease in India by finding *Borrelia* in blood smear of a 15 year old boy in Shimla. So far, no data on seroprevalence rate in India is available.

Overall, 13% of the population in our study was seropositive. The seroprevalence rates in USA, Europe and Japan have been reported to be 2-12%, 26%, and 5-21% respectively [6-8,11]. The seroprevalence rate reported in China is 5.06-26.2% [12,13] and in other Southeast Asian countries like Malaysia, it is 4.1% [8]. Arunachal Pradesh which borders with China shows a higher positivity rate as compared to other states (17.8% vs 8.46-9.6%). The seroprevalence rate is lower in European countries. In Greece the IgG positivity is 3.27% [14], where as in Netherland and Slovenia the incidence of Lyme disease is 103 and 206 per one lakh population [15].

Higher prevalence rate was observed in females (15.86% vs 10.95%). This may be due to the fact that

in North-Eastern region, females work more and are frequently exposed to the jungle environment, thus exposing them to tick bites. Higher IgG positivity rates were seen in the age group of 16-30 years in both sexes suggesting that active working population is at higher risk of acquiring the disease. Similar finding has been reported by Steere et al [16], where the mean age group was 28 years. Majority of the service personnel included in the study, were recruits from the regimental centres and belonged to the North-Eastern region. Additional 132 samples (not included in the 500 subjects) from troops not belonging to North-Eastern states but serving in that region, were also tested for IgG to *B burgdorferi*. Only two subjects were found positive. This may be due to the fact that troops are well clothed while operating in this area, thus affording protection from tick bites. In this study, kits with recombinant antigens specific to *Borrelia burgdorferi* were used, hence, the chance of cross-reaction with other *Borrelia* species was ruled out [17]. As some false positivity has been reported with syphilitic sera and sera containing ANA, we carried out these tests including RF in the samples which were positive for IgG antibody. To evaluate the kit for cross-reaction, we also tested 10 known syphilitic sera for IgG antibody to *Borrelia burgdorferi* and all were found negative.

Arunachal Pradesh showed higher prevalence (17.8%) as compared to other North-Eastern states due to dense vegetation/jungle in this state.

A high (13%) seroprevalence rate suggests that Lyme disease exists in India. In North India a study carried out by Handa et al [18], among patients with monoarthritis showed one positive case with IgG antibody out of 27 but they could not detect any positivity in 12 blood donors. This may be due to use of only North-American strain 2591 as an antigen. In our study we used multiple antigens of strains from different geographical regions. Further study in other parts of the country and detection of both IgG and IgM are required in suspected cases for accurate diagnosis. We could not carry out a survey of the tick population, which may be necessary to find out the species of vector involved in transmission of the *B burgdorferi* in India.

Table 1

Age and sex wise distribution of IgG sero-positivity to *Borrelia burgdorferi*

Sex	Total tested	0-15 years	16-30 years	31-45 years	>45 years	Total
Male	292	0/10 (0%)	14/122 (11.48%)	12/121 (9.92%)	6/39 (15.38%)	32/292 (10.95%)
Female	208	0/11 (0%)	20/107 (11.48%)	10/68 (9.92%)	3/22 (15.38%)	33/208 (15.86%)

Table 2

State wise distribution of study group and their sero-prevalence

State	Total	IgG positivity to <i>Borrelia burgdorferi</i> (%)
Arunachal Pradesh	230	41 (17.8)
Meghalaya	88	8 (9.09)
Nagaland & Manipur	130	11 (8.46)
Assam	52	5 (9.6)

Conflicts of Interest

This study has been funded by the research grants from the office of Director General Armed Forces Medical Services.

References

1. Steere AC, Malawista SE, Snyderman DR, et al. Lyme Arthritis: An epidemic of oligoarticular arthritis in children and adults in three Connecticut communities. *Arthritis Rheum* 1977; 20 : 7- 10.
2. Steere AC, Broderick IE, Malawista SE, et al. Erythema Chronicum Migrans and Lyme Arthritis: Epidemiologic evidence for a tick vector. *Ann J Epidemiol* 1978; 108: 312-5.
3. Steere AC. Lyme disease. *N Engl J Med* 1989; 321:586-9.
4. Hauser U, Lehnert G, Lobentanzer R. Interpretation criteria for standardization of western blots for three European species of *B burgdorferi* sensu lato. *J Clin Microbiol* 1997; 35 : 1433-44.
5. Berger BW, Johnson KC, Kodner C, et al. Cultivation of *B burgdorferi* from erythema migrans lesions and perilesional skin. *J Clin Microbiol* 1992; 30: 359-62.
6. Centre for Disease Control and Prevention of Lyme Disease-USA. *MMWR Morb Mortal Wkly Rep* 1995; 45: 481.
7. Fanner H, Vander LS, Sauvien MJ, et al. The prevalence and incidence of clinical and asymptomatic Lyme Borelliosis in a population at risk. *J Infect Dis* 1991; 163: 305-9.
8. Takala N. *B burgdorferi* (Strain *B afzelii*) antibodies among Malaysian blood donors and patients. *South-East Asian J Trop Med Public Health* 2002; 33: 787-93.
9. Geevarghese G, Fernandes S, Kulkarni SM, et al. A check list of Indian ticks (Acari: Ixodoidea). *Indian J Animal Sci* 1997; 67: 17-25.
10. Patial RK, Kashyap S, Bansal SK, et al. Lyme disease in a Shimla boy. *J Assoc Physicians India* 1990; 38:503-4.
11. Steere AC. *Borrelia burgdorferi* (Lyme disease, Lyme Borreliosis). In: Mandell GL, Bennett JE, Dolin R editors. *Principles and Practice of Infectious Diseases*. 5th ed. London: Churchill Livingstone, 2000; 2504-9.
12. Ai CX, Zhang WF, Zhao JM, et al. Sero-epidemiology of Lyme Disease in an endemic area in China. *Microbiol Immunol* 1994; 38 : 505-9.
13. Takala N, Ishiguro F, Fujita H, et al. Lyme Disease Spirochetes in Ticks from Northern China. *J Parasitol* 1998; 84: 499-504.
14. Statnani M, Toto SG, Brawn A, et al. Very low seroprevalence of lyme Borreliosis in young Greek males. *European J Epidemiol* 2000; 16: 496.
15. Smith R, Takkinen J. Lyme borreliosis: Europe wide coordinated surveillance and action needed? *Eurosurveill* 2006; 11(6): E 0600622.1(cited 2006 June 23). Available at <http://www.eurosurveillance.org/ew/2006/060622.asp#1>.
16. Steere AC, Bortenhagen NH, Craft JE, et al. The early clinical manifestation of Lyme Disease. *Ann Intern Med* 1983; 99: 76-80.
17. Rahn DW, Malawista SE. Lyme Disease: Recommendations for diagnosis of Lyme Disease and Treatment. *Ann Intern Med* 1991; 114: 472-6.
18. Handa R, Wali JP, Singh S, et al. A prospective study of Lyme Arthritis in North India. *J Med Res* 1999; 110: 107-9.

ATTENTION ADVERTISERS

Rates of advertisements in Medical Journal Armed Forces India :

	Black & White	Colour	Size
Full page (per issue)	Rs. 5000/-	Rs. 10000/-	25 x 18 cms
Half page (per issue)	Rs. 3000/-	Rs. 6000/-	18 x 12 cms
(Positive for advertisement to be supplied by client)			
Inserts (two pages)	Rs. 5000/- per insert (Black and White / Colour)		
(Printed inserts to be supplied by client)			

Address for Correspondence

EDITOR-IN-CHIEF
MEDICAL JOURNAL ARMED FORCES INDIA
C/O ARMED FORCES MEDICAL COLLEGE, PUNE - 411 040.