Journal of Diabetic Association Medical College 2021;5(2)5-9

Original Article

Study on Sero-prevalence of IgG Antibody of Lyme Disease in Asymptomatic Population at Risk

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Abstract

Background: *Borrelia burgdorferi* infection is the most frequent tick-transmitted disease worldwide.

Objective: The aim was to assess the sero-prevalence of *B. burgdorferi* infection among forestry workers and farmers in Sylhet, the north-east region of Bangladesh.

Methods: This was a descriptive cross-sectional study based on laboratory findings of sero-prevalance survey. Blood samples from 44 forestry workers and farmers were obtained to determine the presence of antibodies to *B. burgdorferi.* The sera were tested by ELISA method. Demographic data regarding residence, age, gender, profession, tick bite history and contact with animals were collected by questionnaire. All results were evaluated statistically using the χ^2 test and Z test.

Results: Sero-prevalence rate was 9.09% for IgG Antibody of *B. burgdorferi*. Sero-prevalence rate of IgG antibody of *B. burgdorferi* in asymptomatic populations are statistically significant ($p<0.05$) in chi-square test. Seropositivity rates were related to age, gender, and common risk factors for the disease. Seropositivity of *B. burgdorferi* suggests infections by the organisms and presence of Lyme disease in our country. Lyme disease may cause severe illness which can be cured by antibiotic treatment. Its high prevalence in this study warrants greater awareness among clinicians so that they can suspect the disease on clinical grounds and either send them for diagnostic evaluation or initiate empirical treatment.

Conclusion: A large scale multilevel study is needed to get a clear picture of sero-positivity of *Borrelia burgdorferi* infection in every region of Bangladesh. Additional public health measures to prevent tick exposure should be explored in this high risk population, and further studies are needed to confirm and extend the sero-prevalence findings.

Keywords: Borrelia burgdorferi, Sero-prevalence, ELISA

Introduction

Lyme disease or Lyme borreliosis is an emerging infectious disease caused by at least three species of bacteria belonging to the genus *Borrelia* [1]. *Borrelia burgdorferi sensu stricto* is the main cause of Lyme disease in the United States, whereas *Borrelia afzelli* and *Borrelia garinii* causes most European & Asian cases [2].

Lyme disease was recognized in 1975 in Lyme country of Connecticut, USA in children suffering from arthritis [3]. The Lyme disease bacterium, *Borrelia burgdorferi*, normally lives on mice, squirrels and other small animals. It is transmitted among these animals and to humans through the bites of certain species of ticks. In the north-eastern and north-central United States, the black-legged tick (or deer tick, *Ixodes scapularis*) transmits Lyme disease. In the Pacific coastal United States, the disease is spread by the western black-legged tick (*Ixodes pacificus*) [4]. In Indian subcontinent the disease is transmitted by the tick *Ixodes ricinus* complex.[5]

Lyme disease is common worldwide and is associated with areas inhabited by ticks [6, 7]. The largest number of cases of Lyme disease has been reported in the USA, where the disease is found in all states. In the Far East (Japan and China), Australia, South America, and Western Africa, the disease is diagnosed less frequently. In Europe, the disease is found in almost every country. According to a WHO report, all of Europe should be regarded as an endemic area of Lyme disease.[7,8]

Since surveillance was begun by the CDC in 1982, the number of cases in the USA has increased dramatically. Prevalence in USA is 2-12%. The infection is also reported

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in Asian countries including China, Korea, Japan, Indonesia, Malaysia, Nepal and India. The highest attack rates are in children from 0-14 years of age and in persons over the age of 30 years.[9]

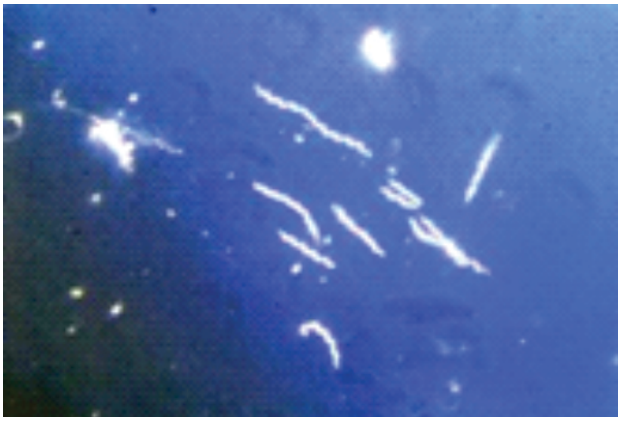

Figure 1: *Borrelia burgdorferi* under microscope (source: CDC)

Figure 2: Blacklegged ticks Lyme disease is common worldwide and is associated with areas inhabited by ticks.[6,7]

Routine diagnosis of Lyme borreliosis in humans is based on the determination of the levels of specific antibodies of the IgM and IgG classes, mainly in blood, cerebrospinal fluid, and synovial fluid. According to guidelines promulgated in the USA and Germany, serological diagnosis should follow the principle of a two-step procedure, with enzyme-linked immunosorbent assay (ELISA) or IFA as the first step; if reactive, this step is followed by immunoblotting (American College of Physicians, 2004; Brouqui et al., 2004; Centers for Disease Control, 1995).

Problems in sensitivity and specificity of ELISA-immunoblot combination for the serological diagnosis of Lyme borreliosis has been highlighted in a recent study (Ang et al., 2011). Moreover, immunoblot is difficult to standardize (Hauser et al., 1998; Hauser et al., 1999) and it has not yet been evaluated on consecutive clinical samples so that whether this approach indeed improves the diagnosis of patients in Lyme disease remains unknown. In the present study we only used ELISA method to explore the seropositivity of *B. burgdorferi*.

High-risk groups include foresters, farmers, ranchers, field biologists, trail builders, trail users, rangers, gardeners, hunters, joggers and vacationers. Other than geography most cases of Lyme disease are thought to result from peri-residential exposure to infected ticks. Persons, who reside, work or recreate in wooded areas or areas of overgrown brush are at risk of acquiring the infection. Several environmental factors have been identified that favour increased tick abundance. Some studies have shown that certain soil types, watersheds and ground cover are associated with tick abundance.[9]There is paucity of data on Lyme disease in India. A sero-prevalence study of *Borrelia burgdorferi* was carried out recently in north eastern states of India. Arunachal Pradesh showed higher sero-prevalence rate (17.80%) as compared to Meghalaya (9.09%), Assam (9.60%), Nagaland (8.46%) & Manipur (8.46%) of India.[5]

A high sero-prevalence rate (17.80%) at least in one state suggests that Lyme disease exists in India. The infection is also reported in other Asian countries including China, Japan, Korea, Indonesia, Malaysia and Nepal. As north-east region (Sylhet) of Bangladesh has borders with Indian states where high sero-prevalence rate is present, so there is a probability of the disease to occur in neighbouring districts of our country.

Literature search failed to reveal any study conducted in this region to explore the sero-prevalence of Lyme borreliosis. All the epidemiological information quoted in relation to *B. burgdorferi* infection so far has been derived from studies carried out in developed countries and some developing countries except Bangladesh. So the incidence in Bangladesh is not known. The present study was undertaken to investigate this uncharted territory of Lyme borreliosis in Sylhet division on a limited scale.

The location, surroundings, and living conditions of the people of Bangladesh seemed indicative of a high risk for tick bites and related infections. This study was carried out to assess the sero-prevalence of *B. burgdorferi* infection in the rural area of Sylhet and to define associated risk factors in the high-risk group.

Materials & Methods

This was a cross-sectional study conducted in the department of Microbiology at Sylhet MAG Osmani Medical College, Sylhet from July 2010 to June 2011. Study variables were IgG antibody of B. burgdorferi, age, sex, occupation, animal handling etc.

Figure 3: Rash & Erythema Migrans (EM)

A total number of 44 subjects were studied. Samples were collected from the forest area of Sylhet division. A total of 5ml of venous blood were taken from each patient with asceptic precautions. Written consents were obtained from all the subjects for inclusion into the study. All information's as per questionnaire were taken. Ethical permission was obtained from the ethical review committee of Sylhet MAG Osmani Medical College beforehand. All the ethical committee guidelines were followed during the conduct of the study. Serum IgG antibodies against B. burgdorferi was measured by the ELISA method for the detection of IgG antibodies to B.burgdorferi in human serum(Manufacturer of the reagent: MAST DIAGNOSTICA, Laboratoriumspraparate GmbH, Reinfeld, Germany). All data were checked and were analysed with the help of SPSS with version 17.0.

Results

Age range of asymptomatic individuals were 13 to 55 years (Mean ± SD= 33.11±11.75).

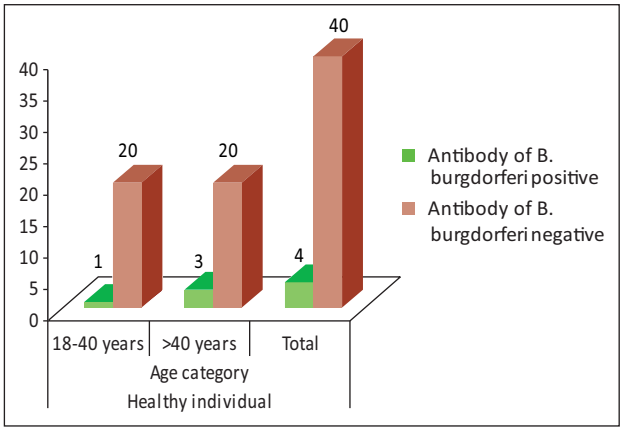

Figure 4.1: Age Distribution of the Asymptomatic Individuals

None was seropositive for *B. burgdorferi* below 18 (eighteen) years of age in the study sample.

Gender distribution of the respondents:

Among the all 44 subjects studied 6 were male and 38 were female.

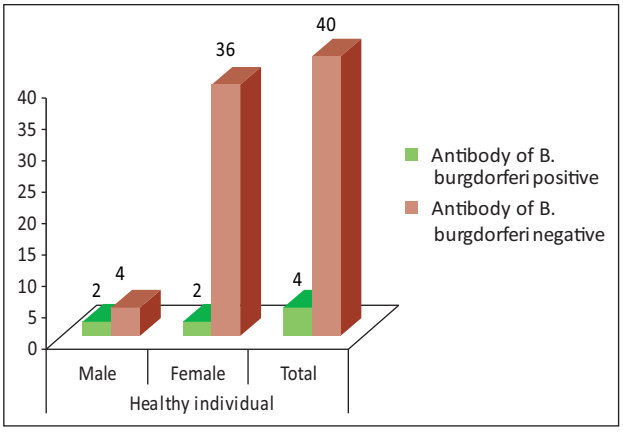

Figure 4.2: Gender Distribution of the Asymptomatic Individuals

Gender distribution in seropositive asymptomatic individual is statistically significant in chi-square test ($p < 0.05$). Higher prevalence rate (33.33%) was observed in males compared to females (5.26%).

Occupation of the Respondents:

Among 44 asymptomatic individuals 41 persons (93 %) were tea garden labour of which seropositive for IgG antibody of *B. burgdorferi* were 3 (7%) and seronegative were 38 person (93%). And 3 persons (7%) were farmer of which seropositivity rates were 1 (33%) and seronegative 2(67 %). This difference was not statistically significant ($p > 0.05$) in chi-square test.

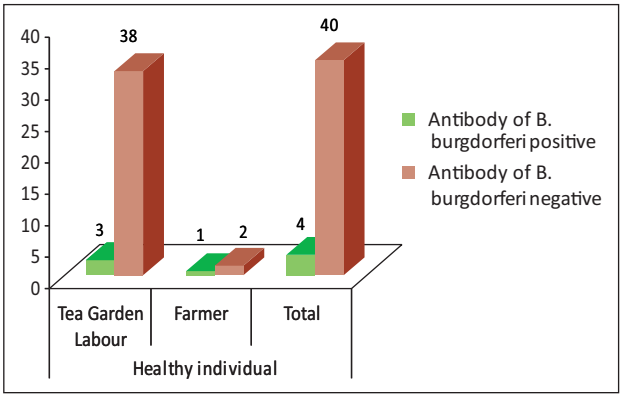

Figure 4.3: Occupation of the Asymptomatic Individuals

Table 1: Relationship of IgG Antibody of *B. burgdorferi* with Occupation of the Respondents

Occupation	Antibody of *B. burgdorferi*		Total
	Positive	Negative	
Tea garden labour	3 (7%)	38 (93%)	41 (100%)
Farmer	1 (33%)	2 (67%)	3 (100%)
Total	4 (9.09%)	40 (90.91%)	44 (100%)

Out of all, highest rate of seroprevalence of IgG antibody of *B. burgdorferi* observed in farmers.

Socioeconomic status of the Respondents:

The subjects were divided into three socioeconomic groups according to their monthly family income. According to the classification of Bangladesh Bureau of Statistics (BBS), 2002, families with monthly income less than Taka 5,000/- were grouped as low, monthly income taka 5,000/- to taka 10,000/- as middle and those earning more than taka 10,000/- per month as high socioeconomic group.

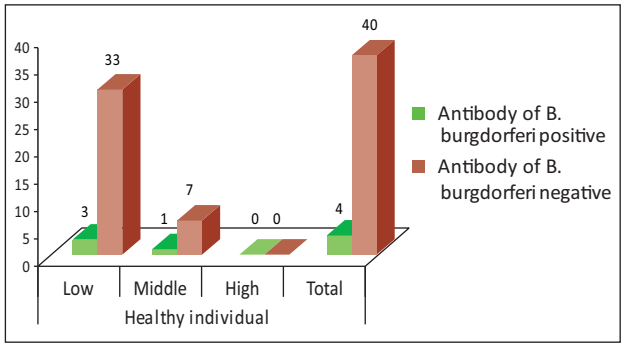

Figure 4.4: Socioeconomic Status of the Asymptomatic Individuals

Majority of the persons came from the low socioeconomic background followed by middle class.

Table 2: Relationship of IgG Antibody of *B. burgdorferi* with Socioeconomic Status

Status	Antibody of *B. burgdorferi*		Total
	Positive	Negative	
Low	3 (8%)	33 (92%)	36 (100%)
Middle	1 (13%)	7 (87%)	8 (100%)

History of animal handling:

About 12 persons gave the history of animal handling of which 2 (16.67%) were seropositive and 10 persons were seronegative for IgG antibody of *B. burgdorferi*. Rest 32 persons had no history of animal handling out of which 2 (6.25%) person were seropositive and 30 were seronegative.

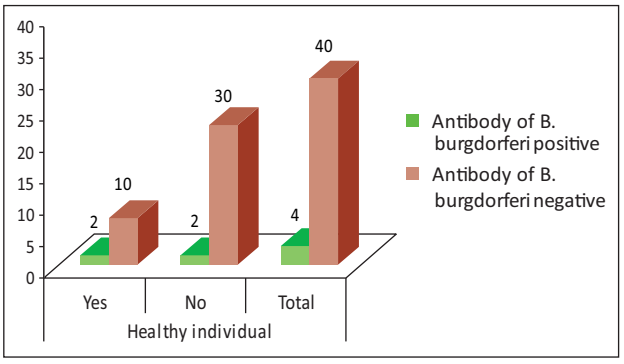

Figure 4.5: History of Animal Handling of the Asymptomatic Individuals

In Chi-square test seropositivity for IgG antibody of *B. burgdorferi* with history of animal handling was found statistically significant ($p<0.01$).

Seroprevalence of IgG antibody among respondents:

A total of 44 persons were studied. Out of the total 44 persons 6 (13.64%) were male, 38 (86.36%) were female. Among the asymptomatic individual 4 (9.09%) was positive for IgG Antibody of *B. burgdorferi*. Sero-prevalence rate of IgG antibody of *B. burgdorferi* in asymptomatic individuals are statistically significant ($p<0.05$) in chi-square test.

Discussion

A sero-prevalence study of *Borrelia burgdorferi* was carried out recently in north eastern states of India. Arunachal Pradesh showed higher seroprevalence rate (17.80%) as compared to Meghalaya (9.09%), Assam (9.60%), Nagaland (8.46%) & Manipur (8.46%) of India. (Jetley et al., 2008). A high seroprevalence rate (17.80%) at least in one state suggests that Lyme disease exists in India. The infection is also reported in other Asian countries including China, Japan, Korea, Indonesia, Malaysia and Nepal. As north-east region (Sylhet) of Bangladesh has borders with Indian states where high seroprevalence rate is present, so there is a probability of the disease to occur in neighbouring districts of our country.

Gender distribution in seropositive asymptomatic individual is significant in chi-square test ($p<0.05$). Higher prevalence rate (33.33%) was observed in males compared to females (5.26%). This may be due to fact that males work frequently to the deep jungle environment thus exposing them to tick bites. A study in North –Eastern states of India, showed that higher positivity rate in female compared to male (15.86%VS 10.95%).[5]

In this study age range of asymptomatic individuals were 13 to 55 years (Mean ± SD= 33.11±11.75). For 18-40 years age the rate was 5 % (out of 20) and for more than 40(forty) years age rate was 15 % (out of 20), where persons with more than 40 years of age showed higher seropositivity rates. A study was done in department of Microbiology in Madrid shows Major prevalence in the age group between

41 to 50 years (22.6%) [10]. Study in North India found, higher IgG positivity rates in age group of 16-30 years. This suggested that active working population is at higher risk of acquiring the disease [5].

This study was done in outdoor workers that include tea garden labour and farmers. Higher prevalence rate (33.33%) was observed in farmers compared to Tea garden labour (7.32%). This may be due to fact that farmers have close contact of their domestic animals and expose with tick bites.

A study in Spain on 302 outdoor workers found 15% (44/302) of them as seropositive [11]. A cross sectional study was done in Poland in 1153 workers exposed to ticks (880 forestry workers, 273 farmers). Researchers in aforementioned study found seroprevalence in farmers 38.3%, and in foresters 28.1% [12].

In our study about 12 persons (27.27%) gave the history of animal handling of which 2 (16.67%) were seropositive and 10 persons were seronegative for IgG antibody of *B. burgdorferi* . Rest 32 persons (72.73%) had no history of animal handling out of which 2(6.25%) persons were seropositive and 30 persons were seronegative. This difference was significant ($p<0.01$) in Chi-square test. Among the asymptomatic individuals four (9.09%) were positive for IgG Antibody of *B. Burgdorferi* . Seroprevalence rate of IgG antibody of *B. burgdorferi* in asymptomatic individuals are statistically significant ($p<0.05$) in chi-square test.

Some studies have shown the importance of the confirmations of ELISA results by western blot (WB), especially in evaluating patients with clinical symptoms. In those studies patients who were seropositive by both ELISA and WB had some potentially related health complaints, as well as histories of tick bite and animal contact [13].

Conclusion

The study group consisted of forestry workers & farmers in the rural area of Sylhet division. The location, surroundings & living conditions of the people seemed indicative of a high risk for tick bites & related infections. The questionnaires were evaluated to investigate the effects of factors that might increase the risk for the acquisition of the infections, e.g. age, gender, profession, tick bite history, contact with animals and symptoms related to Lyme borreliosis. The seroprevalence of *B. burgdorferi* was 9.09% in forestry workers & farmers. Seropositivity of *B. burgdorferi* suggests infections by the organisms and presence of Lyme disease in our country. Further work is needed to judge the best test for screening & diagnosing active infection. Lyme disease may cause severe illness which can be cured by antibiotic treatment. Its high prevalence in this study warrants greater awareness among clinicians so that they can suspect the disease on clinical grounds and either send them for diagnostic evaluation or initiate empirical treatment.

Recommendations

- A large scale multilevel study is needed to get a clear picture of seropositivity of *Borrelia burgdorferi* infection in every region of Bangladesh.
- Further population & vector biology studies are required to find out the exact species involve in transmission of the organism in addition to confirm and extend the sero-prevalence findings.
- Additional public health measures to prevent tick exposure should be explored in this high risk population, One goal of future investigations will be to construct a map showing the distribution of the disease to allow the identification of high risk areas.

Conflict of interest: No

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