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Seroprevalence of Lymes disease in the Nagarahole and Bandipur forest areas of South India

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Purpose: The aim of this study was to determine the seroprevalence of Lymes disease in a population at risk in south India. **Methods:** Prospective ongoing study and included screening of forest workers and staff of Nagarahole and Bandipur forest ranges in South India for Lymes disease. Screening included a detailed questionnaire for Lymes disease, complete ocular and systemic examination by an ophthalmologist and infectious disease specialist and blood collection. ELISA for IgM and IgG antibodies for *Borrelia burgdorferi* were performed on the collected sera samples. Western blot confirmation was done on the seropositive samples. Ticks were also collected from these forest areas for future studies to detect if they harbor *B. burgdorferi.* **Results:** Seroprevalence of 19.9% was noted by ELISA. Western blot confirmation was seen in 15.6% of the seropositive samples. There was significant correlation between seropositivity and exposure to tick bites ($P = 0.023$). **Conclusion:** There is a high seroprevalence of infection with *B. burgdorferi* in the forest areas of Nagarahole and Bandipur ranges in south India.

Key words: Enzyme linked immunosorbent assays, India, Lymes disease, Seroprevalence, Western blot

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Lymes disease is caused by a spirochaete *Borrelia burgdorferi.* It is transmitted by the bite of infected ticks belonging to the genus Ixodes (deer ticks). The disease is characterized by heterogenous manifestations, overlapping symptoms, and high antigenic variability making the diagnosis difficult. The early phase is characterized by erythema migrans and musculoskeletal symptoms. The late phase is characterized by involvement of nervous system, heart, joints, eyes, and chronic skin changes. If detected in the early phase, it is completely treatable with antibiotics. Late, delayed or inadequate treatment may lead to serious complications.[1] The current definition of Lymes disease includes a patient who develops erythema migrans (EM) within 30 days of exposure in an endemic area or endemic area exposure, without EM, but with signs involving one organ system and a positive laboratory test or no history of exposure, but with EM as well as involvement of two organ systems or no exposure in an endemic area, but with EM and a positive serology.[2] The diagnosis involves a two-step testing initially by enzyme linked immunosorbent assays (ELISA) for IgG and IgM antibodies followed by a western blot confirmation.[1]

Lymes disease is endemic in the United States and has also been reported from Europe and Australia. In India, Lymes disease is not commonly considered as a differential diagnosis of uveitis. In 2009, we published the first serological and western blot confirmed case of neuroretinitis due to Lymes disease from the forest areas of Nagarahole and Bandipur in south India.[3] Since then we have had two more similar cases from these areas. There has also been an increase in sporadic reports of Lymes disease from India in recent years.[4-13]

Currently, we do not have any data on the seroprevalence of this disease in our patient population nor do we know if Lyme disease is underdiagnosed in India. The aim of this study was to determine the seroprevalence of Lymes disease in a population at risk in South India and if Lymes disease is under diagnosed in India.

Methods

A prospective study was undertaken in March 2017 and the second phase of this study is still going on. Phase 1 of this study was to find the seroprevalence of Lymes disease in a patient population at risk in South India. The population

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screened included forest workers and staff of the Nagarahole and Bandipur forest ranges in South India. These forest areas were chosen as our initial cases were from these areas.

Inclusion criteria

Subjects ≥18 years of age, working or living in the forest areas of Nagarahole and Bandipur ranges, and those patients capable of giving informed consent were included in the study.

Forest workers and staff were screened in different areas of these ranges in south India. After an informed consent, all participants completed a detailed questionnaire about age, gender, profession, exposure to tick bites, area of residence, symptoms including constitutional symptoms such as fever, malaise and flu like symptoms, skin rashes, eye involvement, nervous system, and joint-related symptoms. Ocular evaluation was done by an ophthalmologist and included best corrected visual acuity, slitlamp, dilated fundus, and intraocular pressure examinations. Extraocular motility and pupillary examinations were done by means of a torch light. Systemic examination was done by an infectious disease specialist.

Blood sampling

About 3.5 mL of peripheral venous blood was collected from the antecubital vein of the subjects after an informed consent. The blood was allowed to clot and the serum was separated by centrifugation. The sera were then transported on dry ice to the base hospital and stored in multiple aliquots at −70°C. All sera samples were screened using an ELISA test for detection of IgM and IgG antibodies for *borrelia burgdorferi* (NovaLisa ™ Borrelia burgdorferi IgM and IgG, Novatech Immunodiagnostica GmBH) and results were interpreted as per manufacturer's instructions. If positive, the samples were tested by western blot technique for confirmation (AESKUBLOT borrelia-G/-M, AESKU diagnostics, Germany). The interpretation of the results was done as per manufacturer's instructions. Seropositivity was defined as positivity of IgG, IgM, or both on ELISA at a given point of time. About 10% of the seropositive samples were tested for rheumatoid factor, Venereal disease research laboratory test (VDRL), and antinuclear antibodies to rule out cross reactivity.

Phase 2 of the study included collection of ticks from these forest areas to verify if they harbor *B. burgdorferi.* Around 800 ticks were collected from these forest areas by the "tick drag method" and stored at −70°C for further tests such as polymerase chain reaction (PCR) and genome sequencing on these ticks to detect if these ticks harbor *B. burgdorferi*.

Statistical analysis including descriptive statistics was done using SPSS package at <5% level of significance. Chi-square tests were done for comparison of categorical variables with seropositivity.

This study had our institutional ethics committee approval and all necessary clearances from the principal conservator of forests, Karnataka. The study on human subjects was conducted as per the declaration of Helsinki.

Results

About 518 forest workers and helpers were screened. About 472 blood samples with informed consent were taken. The mean age was 36.42 years (18–76 years). There were 425 males (90%) and 47 females (10%). Overall positivity on ELISA was seen in 94 cases (19.9%). In 61 cases (12.9%), the results were indeterminate and negative in 317 cases (67.2%). Confirmation by Western blot was noted in 14 out of 90 seropositive cases (3.2% overall or 15.6% of seropositive samples). There was significant correlation between seropositivity and occupation especially those who were field workers (0.024). There was also significant correlation between seropositivity and exposure to tick bites (0.023). Table 1 shows the statistical correlation between seropositivity and different variables. The seropositive results were classified into four groups: 1) recent *B. burgdorferi infection* with previous exposure (IgM+, IG+) in six cases (1.46%); 2) recent *B. burgdorferi i*nfection only (IgM+, IgG−) in 73 cases (17.76%); 3) past *B. burgdorferi* infection (IgM−, IgG+) in 15 cases (3.65%), and 4) no *B. burgdorferi* infection (IgM−, IgG−) in 317 cases (77.13%). Table 2 shows the western blot confirmation in the different groups, exposure to tick bites, and presence of skin rashes with flu like symptoms in different groups. Table 3 shows the presence of constitutional, joint, neurological, and ocular symptoms in

Table 1: Shows the statistical correlation between seropositivity and different variables

Variables	Seropositivity, *n* (%)	Level of significance
Gender	94	0.156
Occupation (field workers)	73 (89)	**0.024**
Fatigue/tiredness	22 (23.4)	0.340
Joint pains/swelling	30 (31.9)	0.657
Tingling, burning, numbness in extremities	11 (11.7)	0.790
Disturbed sleep	10 (10.6)	0.414
Shortness of birth or cough	9 (9.6)	0.814
Palpitations	8 (8.5)	0.779
Headaches	9 (9.6)	0.102
Neck stiffness	3 (3.2)	0.986
Facial paralysis	1 (1.1)	0.665
Blurred vision or redness in eye	23 (24.5)	0.922
Hearing abnormalities	7 (7.4)	0.533
Low grade fever	4 (4.3)	0.260
H/o exposure to tick bites	58 (62.4)	**0.023**
H/o skin rashes with flu like symptoms following tick bites	26 (27.7)	0.937

Table 2: Shows the western blot confirmation in the different groups, exposure to tick bites, and presence of skin rashes with flu like symptoms in different groups

Results	*n*	Percentage	Western blot positive (*n*)	Exposure to tick bites	Skin rashes with flu like symptoms
IgM+, IgG+ (recent infection with previous exposure)	6	1.46	2	5 (83.3%)	0
IgM+, IgG− (recent infection)	73	17.76	10	44 (61.1%)	22 (30.1%)
IgM−, IgG+ (past infection)	15	3.65	2	9 (60%)	4 (26.7%)
IgM−, IgG− (no infection)	317	77.13	-	236 (74.4%)	89` (28.1%)

Table 3: Presence of constitutional, joint, neurological, and ocular symptoms in the different groups

Results	Fatigue and easy tiredness	Joint pains/ swelling	Tingling, burning, numbness in extremities	Blurred vision or redness in eye	Fever
IgM+, IgG+	2 (33.3%)	2 (33.3%)	1 (16.7%)	1 (16.7%)	1 (16.7%)
IgM+, IgG−	17 (23.3%)	21 (28.8%)	9 (12.3%)	21 (28.8%)	3 (4.1%)
IgM−, IgG+	3 (20.0%)	7 (46.7%)	1 (6.7%)	1 (6.7%)	0
IgM−, IgG−	60 (18.9%)	109 (34.4%)	34 (10.7%)	76 (24%)	24 (7.6%)

Table 4: Shows review of literature of Lymes disease from India

References/year	Demographic parameters/type of study	Location	Clinical presentation	Method of diagnosis
Handa *et al*., 1999	1 out of 64 patients Prospective study	North India	Mono/oligoarticular arthritis, blood donors, RA	ELISA (NA2491) 1-false positive
Patial *et al*., 1990	15-year-old male Case report	North India (Shimla)	Meningitis, arthritis, carditis	Detection of *Borrelia* in peripheral blood smear
Praharaj *et al*., 2008	65 out of 500 (13%) Prospective study	North east India	Asymptomatic general population including service personnel and family	Detection of IgG antibodies by ELISA (multiple antigens of several strains)
Babu *et al*., 2010	45-year-old lady Case report	South India (Nagarahole)	Tick bite, neuroretinitis	ELISA (IgM and IgG) Western blot confirmation
Rajeev, 2013	5 cases/newspaper report	Wayanad, south India	History of tick bite, flu like symptoms	ELISA (details not available)
Jairath *et al*., 2014	7-32 years (3:2) - 5 cases; Case series	North India (Harayana)	ECM in all, local lymphadenopathy (3), constitutional symptoms (4)	ELISA (IgM and IgG) Western blot confirmation
Kandhari *et al*., 2014	11-year-old male, German descent Case report	North India	camping trip to west Virginia - skin lesions in scrotum	ELISA (IgM and IgG) Western blot confirmation
Shenthar *et al*., 2014	13-year- old male Case report	South India	Travel to North America - carditis	ELISA (IgM and IgG) With high titres
Bhat *et al*., 2015	2 siblings Case report	North India	Tick bites, neurological, with cranial nerve palsies	ELISA IgM Western blot confirmation
Sharma *et al*., 2017	10-year-old male Case report	North India (Himachal Pradesh)	Traveled to forest area - characteristic ECM on leg and thigh	ELISA IgM +, IgG-
Guliani *et al*., 2017	25-year-old lady Case report	North India	Visit to Himalayas - neuroretinitis	ELISA IgM+, IgG−Western blot confirmation
Tevatia *et al*., 2018	20-year-old male Case report	North India	Headache, quadriparesis, lymphenopathy, hypocellular bone marrow, pulmonary symptoms	Confirmed twice by serology Clinical improvement with doxycycline

the different groups. Migratory joint pains was seen in one out of seven cases (1.1%). Bell's palsy was seen in one out of three cases (1.4%) and both were seen in the group with recent infection. Retinal vasculitis was seen in one case (1.1%) in the group who did not have infection. About 10% of seropositive samples were tested for cross reactivity and were negative for VDRL, rheumatoid factor, and antinuclear antibodies.

Discussion

Praharaj *et al*.[4] noted a seroprevalence of 13% in northeastern states of India and as high as 18% in states like Arunachal Pradesh. The population screened by them included asymptomatic service personnel and their families and tests included detection of IgG antibodies by ELISA in this study.

The authors attributed the increased seroprevalence of infection due to the presence of the Ixodes ticks in the Himalayas. Since our report in 2009,[3] there was a case series of similar symptoms from the adjacent Wayanad district in kerala, South India.[5] Jairath V *et al*.[6] reported five cases of western blot confirmed Lymes disease with characteristic erythema chronic migrans, local lymphadenopathy, and constitutional symptoms and good response to doxycyline. Since then there are multiple reports of Lymes disease from India.[7-13] Table 4 shows review of literature of Lymes disease from India.

In this prospective study, there is a high seroprevalence (19.9%) of *B. burgdorferi* infection in the Nagarahole and Bandipur forest areas in south india. Cross reactivity on serological tests may take place with other bacteria (*Treponema pallidum*, *Treponema pertenue*, nonpathogenic Borrelia, and Leptospira), during certain viral infections that invoke production of polyclonal antibodies such as Epstein–Barr virus, certain autoimmune diseases (rheumatoid arthritis and systemic lupus erythematosus) and in liver disease (hepatitis C). 10% of our seropositive samples tested for cross reactivity were negative for VDRL, rheumatoid factor, and antinuclear antibodies.

The western blot or immunoblot enables detection of antibodies against individual components of the organism and thus provides more information than a whole cell-based ELISA regarding which antigens of *B. burgdorferi* react with serum antibodies. Western blots can be performed to detect either IgM or IgG antibodies. In the Western blot assay, a mixture of bacterial antigens from one or more species enriched with recombinant antigens is subjected to electrophoretic separation which allows isolation of antibodies against individual antigens and arrangement according to their molecular weight. This test has a high specificity and forms the next level of testing after ELISA. Western blot confirmation was noted in 15.6% of our seropositive samples.

Incidentally, Sadanandane *et al*.[14] in their study on prevalence and spatial distribution of Ixodes tick populations in the forest fringes of western ghats reported the presence of Ixodes species of ticks (0.31%). The areas were the Ixodes ticks were seen were close to the forest areas where we screened the forest workers in our study. Our ongoing phase 2 studies on PCR on the collected ticks from these forests, combined with next generation sequencing based molecular approach[15] would yield more information on the presence of *B. burgdorferi* in these ticks.

So how do we interpret the current seroprevalence results of this study in a clinical scenario? This is fairly new information as we do not routinely test for Lymes disease in India. The high seroprevalence suggests the presence of *B. burgdorferi* infection in this population. As a result, it is important to include Lymes serology in our investigative panel especially if the patient is from a population at risk, with history of tick exposure, has characteristic symptoms and signs of Lymes disease. A positive serology in such a scenario will require appropriate treatment with antibiotics.

Conclusion

There is a high seroprevalence of infection with *B. burgdorferi* in the forest areas of Nagarahole and Bandipur ranges in south India. A high degree of suspicion for Lymes disease is required even in India, especially in a patient coming from a population at risk, history of tick exposure, with characteristic signs and symptoms, and a positive serology.

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Declaration of patient consent

The authors certify that they have obtained all appropriate patient consent forms. In the form the patient(s) has/have given his/her/their consent for his/her/their images and other clinical information to be reported in the journal. The patients understand that their names and initials will not be published and due efforts will be made to conceal their identity, but anonymity cannot be guaranteed.

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Conflicts of interest

There are no conflicts of interest.

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