



Lyme disease: Emerging and Re-emerging Metazoonoses of Global Importance

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ABSTRACT

Lyme borreliosis is a tick-transmitted multisystem inflammatory disease caused by the spirochete *Borrelia burgdorferi*. Lyme disease has a worldwide distribution including India. Warmer weather expected to be increase emergence of common vector-borne diseases worldwide without an exception of Lyme disease. Several hundred cases are reported each year. About 10% of cases involve the more serious symptoms of arthritis and 5% experience facial paralysis, in addition to the skin rash that is characteristic of the disease. There is paucity of data on Lyme disease in India. Some study was under taken to study the seroprevalence of *Borrelia burgdorferi* infection in north eastern parts of India. Therefore, India represents a particularly interesting scenario for the study on Lyme disease. Vaccines against the condition are still not very successful. Hence, the importance of recognizing the cutaneous manifestations early, to prevent systemic complications which can occur if left untreated, can be understood. Here in; we review the problems and opportunities for the incidence of Lyme disease in India. Control measures are discussed in the review in the area of ticks and tick-borne zoonotic diseases (TTBDs). The disease warrants constant monitoring and surveillance because once introduced to human population it would be a herculean task to eradicate it.

Keywords: *Borrelia burgdorferi*, Lyme disease, Ticks and Tick-borne diseases (TTBDs)

Rapid changes in the environmental factors like, warmer temperature and altered rainfall patterns are likely to increase the range and burden of disease transmitting vectors, resulting in the emergence of vector-borne diseases. Lyme disease is recently recognized as an important emerging tick borne metazoonoses caused by obligate, Gram negative spirochaete, *Borrelia burgdorferi* (Kurtenbach *et al.*, 2006). Though very commonly reported from the temperate regions across the globe, the incidence has increased worldwide due to increasing travel and changing habitats of the vector. There is paucity of data on Lyme disease in India,



however few cases have been reported from the Indian subcontinent by Vasudevan and Chatterjee (2013). Seropositivity to *B. burgdorferi* infection in northeastern parts of India suggests presence of Lyme disease. The distribution and vector biology studies of *Ixodid* tick in Himalayan region, Arunachal Pradesh, Himachal Pradesh, Assam, Nagaland, Manipur, Meghalaya, Sikkim, U.P. and West Bengal are required to find out the exact species involved in transmission of the organism (Praharaj *et al.*, 2008).

Lyme disease is a silent plague of the 21st century, infecting unsuspecting individuals at an alarming rate, few are known and many are unaware of this growing threat. Lyme disease infections are on the rise in the past 20 years and are threatening to increase in the near future (Bradford and Allen, 2005). Further, the disease is wide rapidly spreading as world in converting into a global village. Hence the importance of recognizing the cutaneous manifestations early, to prevent systemic complications which can occur if left untreated, can be appreciated.

HISTORY

The data regarding this infamous pathogen suggests the various important events in history briefed below.

1883	German physician Alfred Buchwald reported first finding of Lyme borreliosis
1974	Disease was first recognized in the U.S. in New England town of Lyme, Connecticut in a 14-year old boy (Steere <i>et al.</i> , 1997)
1910	Arvind Afzelius described an amular skin lesion caused by bites of Ixodes tick.
1975	Disease first studied by Dr. Allen Steere, following a mysterious outbreak of juvenile rheumatoid arthritis
1983	Etiological agent was named <i>Borretia burgdorferi</i> .
1980	Etiological agent discovered by Willy Burgdorfer.
1990	Patial and coworkers reported case of Lyme disease in Shimla (India).
1996	Aberer identified <i>Dorrellal</i> structures in skin biopsies of erythema chronicum migrans.
1999	Brorson demonstrated transversion of oystic forms of <i>B. burgdorferi</i> to normal mobile spinohaetes
2003	Bradford Research Institute produced High Resolution Microscope Imaging of Lyme spirochets and cyst forms

ETIOLOGY

Lyme disease is caused by the spirochete, *Borrelia burgdorferi*. This spirochete possesses several morphological, structural, ecologic and genomic features that are distinctive among prokaryotes (Walker, 1998). This spirochete is 11-39 micrometers long and 0.3-0.4 micrometer wide. The spirochete is highly flexible, has 7-11 flagella and moves by rotation and twisting (Nardelli *et al.*, 2008). They survive in liquid environment such as mud water or blood. The medium used to grow *Borrelia burgdorferi* is called Barbour-Stoenner-Kelly (BSK) medium (Bradford, 2004). The organism possesses 3-7 plasmids, one of which codes for two major proteins 31kDa OSPA protein and 34kDa OSPB protein. These proteins

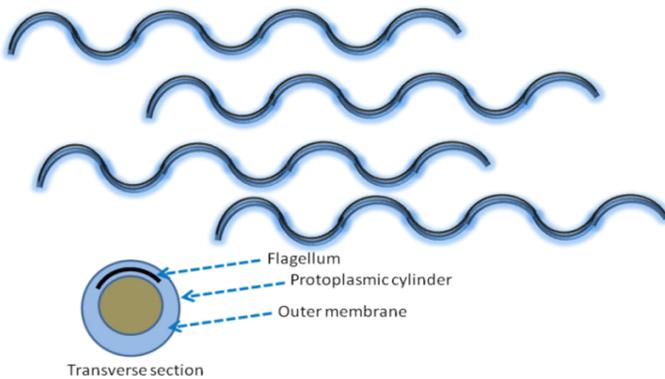


Fig : Schematic structure of *Borrelia* spp.

Fig 1: Schematic diagram showing structure of *Borrelia burgdorferi* (Bradford, 2004)

Table1: Worldwide geographical distribution of *Borrelia* species

Species	Geographic distribution
<i>B. burgdorferi</i>	Europe and North America
<i>B. garinii</i>	Europe and parts of Asia
<i>B. afzelli</i>	Europe and parts of Asia
<i>B. andersoni</i>	North America
<i>B. valaisiana</i>	Central Europe, UK, Ireland
<i>B. lusitaniae</i>	Portugal, Tunisia
<i>B. japonica</i>	Japan
<i>B. sinica</i>	China
<i>B. bissettii</i>	Slovenia and North America



appear to undergo antigenic variation during the course of infection (Bradford, 2004).

The *Borreliae* causing Lyme disease is divided into several genospecies, three of which have been firmly established and are well accepted as a human pathogen they are worldwide in distribution (Hildenbrand *et al.*, 2009).

IMMUNOLOGY

The outer membrane of *Borrelia burgdorferi* is composed of various unique outer surface proteins (Osp) that have been characterized (OspA and OspF). The fragment of the spirochete membrane with incorporated DNA is known as a “bleb”. The function of blebs is to bind IgM antibodies thereby protecting the organism from the immune system. Bb is one of the most immuno-suppressive infectious agents, affecting cellular immunity, humoral immunity, and natural killer (NK) cell population. Spirochetes in general are difficult to treat for several reasons. Since they have the ability to burrow into or between cells and hide, gaining protection from the immune system, has highly unusual outer membranes and the molecular architecture of these membranes is responsible to cause persistent infection.

Table 2: Distinguishing characteristics of *Borrelia burgdorferi* (Bradford, 2004)

Distinguishing characteristics of <i>Borrelia burgdorferi</i>			
Sr.No	Distinguishing characteristics of <i>Borrelia burgdorferi</i>		
1	Internal Flagella	6.	Cellular Invasion Ability
2	Glycoprotein Coat	7.	Cyst Formation
3	DNA Net Arrangement	8.	Internal Antigenic Proteins
4	Bleb Formation	9.	Surface Antigen Transformation
5	Prolonged Replication Time	10.	Spiral Shape

EPIDEMIOLOGY

In India first case reported by finding *Borrelia* in blood smear of a 15 year old boy in Shimla (Patial *et al.*, 1990). In India females showed higher positivity rate as compared to males (15.86% v/s 10.95%) in North-Eastern region. The higher prevalence rate in the age group of 15-30 years in both sexes (11.48% in male and 18.69% in female). Major states affected Arunachal pradesh, Nagaland, Manipur and Assam. In north India, a study carried out by Handa and coworkers (1999) among patients with monoarthritis showed positive cases with IgG antibody. In India, there is lack of survey of the tick population, which is necessary to find out the species of vector involved in transmission (Praharaj *et al.*, 2008).

Table 3: IgG positivity to *Borrelia* species in North-East region in India

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

Retrospective case report from south India emphasizes the significance to include the lyme diseases in the differential diagnosis of neuroretinitis, especially if the patient hails from a forest area (Babu and Murthy, 2010). The *Ixodes* ticks have been identified in the Himalayan region of India. The Centre for Disease Control and Prevention (CDC) carried out surveillance for Lyme disease in India in 1982 and 1991, and Lyme disease was classified as a reportable disease (Geevarghese *et al.*, 1997).

HOST/RESERVOIRS

Approximately 40 species of mammals and birds have been recognized as a reservoir for *Borrelia burgdorferi* (Gern *et al.*, 1998). The transmission of *Borrelia* occurs from *Ixodid* ticks to dogs, cats and human. Moreover, various researchers have proposed the dog as ‘sentinel animal’ for the detection of emerging risk areas of Lyme disease (Merino *et al.*, 2000; Bhide *et al.*, 2002). Mice, squirrels and other small rodents, deer, sheep, cattle and many bird species act as hosts (Burgdorfer, 1983). The higher seroprevalence (33%) in the domestic cats include importance of this pet animal in Lyme disease epidemiology. Foresters, farmers, campers and nature enthusiasts are groups considered at a risk of infection due to their increased outdoor exposure. The age distribution of Lyme disease is bimodal, with the highest number of cases occurring in children aged from 5 to 14 years, and in adults aged from 55 to 70 years (Bacon *et al.*, 2008).

TRANSMISSION

In most European and Asian countries the tick, *Ixodes ricinus*, most important vector. Human get the infection by the bite of infected tick. The other ways of transmission are transfer across placenta to developing fetus or through breast milk, blood transfusion. The disease agent has been isolated from urine and colostrums in cattle, dog and mice suggesting that these fluids may be possible contagious route (Bhide *et al.*, 2004).



PATHOGENESIS

Ticks, those are harboring the organism in their tissues, transmit disease to susceptible host during hematophagy. At the site of bite, organism causes severe inflammation and further disseminates to other parts of body via blood and/or lymph. To reach predilection sites such as heart, connective tissue and different joint modules including ligaments, tendons; organism leaves blood by penetrating endothelial wall and gains access towards extracellular matrix which helps evade host's immune components too.

Borellia uses several mechanisms to evade the immune system and may stay dormant for years in tissues. First, it chooses to localize in the extracellular matrix which comparatively has less immune cell accessibility than blood and lymph do. Second, it has capacity to alter its outer membrane structure mainly a protein named Osp protecting organism from antibody attack. Third, it can manipulate host's immune response in several ways by altering secretion of various cytokines which are the main key components to organize the action of both innate and adaptive arms of immune response.

The spirochaete survives in a dormant state in the nymphal tick midgut during which it expresses primarily OspA. The tick feeds for several days on a mammal (early summer), OspC replaces OspA. Within days after disease onset, most patients have an IgM antibody response to OspC or the flagellar protein of the spirochaete. If early stage left untreated, in several weeks after the rash, the bacterium and its effects can spread throughout the body to affect the joints, heart and nervous system (Tilly *et al.*, 2008). Serum resistance and complement activation also play important roles in pathogenesis. Infection leads to expression of lipoproteins which in-turn activates various inflammatory cells and mediators (Brade *et al.*, 1992).

Evidences suggest that the immune-dominant epitopes of organism are present at inner aspect of cell membrane which is quite inaccessible for white blood cells. Organism is also capable of forming sturdy cysts in tissues during nutrient scarcity (Bradford, 2004).

Clinical Features in Humans

The interplay between various spirochaetal factors (e.g. infection with different genospecies) and host factors (e.g. cytokine patterns at the site of infection) influences the course of disease. The manifestations of Lyme disease in humans are generally categorized into three entities namely localized, disseminated and persistent infection. Localized and disseminated part of infection is component of early phase while persistency is its late counterpart (Mulleger, 2004).

Early localized Lyme disease

It is characterized by erythema migrans, a distinguishing rash of few to several centimeters which is considered as pathognomonic for Lyme disease. More than 80 % of affected cases show this condition. These lesions are known as “dermatoborreliosis” (Melski, 1999). Another type (about 20% cases) is multiple lesions due to hematogenous spread. CBCL (Cutaneous B-cell lymphocytoma) is a least observed manifestation of dermatoborreliosis (about 5% cases). Several reports have suggested the link between CBCL and borrelia infection (Goodlad *et al.*, 2000).

Early Disseminated Stage

These occur within weeks or months after tick bite. The acute neurological manifestations are frequently observed compared to its chronic counterpart. Acute form shows painful neuropathy syndrome also known as Bannwarth’s syndrome, which is frequently shown by the adults compared to children. Other signs such as lymphocytic meningitis, facial palsy (Bell’s palsy) and peripheral neuropathy present. The cardiac symptoms include conduction defects, myopericarditis, cardiomyopathy. Conjunctivitis, joint and muscle pain can be experienced by the patient along with low grade fever and regional lymphadenopathy (Melski *et al.*, 1993).

Chronic or Late Lyme disease (Acrodermatitis chronica atrophicans)

‘Acrodermatitis chronica atrophicans’ is the characteristic manifestation of late Lyme borreliosis (Berger, 1989). Lesions occur particularly in the skin of extremities due to cooler temperatures. Musculoskeletal manifestations include chronic Lyme arthritis, neurologic manifestations include encephalomyelitis and cutaneous manifestations show Lymphocytoma. It occurs primarily in elder age group due to persistent borrelial infection (Silberer *et al.*, 2000). Squamous cell carcinoma and sarcoma can develop in the lesions (Leverkus *et al.*, 2008). *B. burgdorferi* can be cultured from ACA lesions up to 10 years after onset (Asbrink *et al.*, 1985).

Other Manifestations

Eosinophilic fasciitis, (Hashimoto *et al.*, 1996) benign lymphocytic infiltration (Abele *et al.*, 1989), granuloma annulare (Aberer *et al.*, 1999), erythema multiforme (Lesire *et al.*, 2000), urticaria, urticarial vasculitis (Olson and Esterly, 1990) infantile papular acrodermatitis (Baldari *et al.*, 1996) and panniculitis have all been mentioned in few case reports as being associated with *Borrelia* infection.



Clinical Features in Animals

Dogs, cattle, horses and sheep may develop systemic disease, with articular and cardiac manifestations similar to that seen in humans. In horses and cattle symptoms noted are lameness, stiff joints, refusal to feed, depression, laminitis, blindness, spontaneous abortion and encephalitis (Bhide *et al.*, 2004).

Clinically disease is manifested by neurological, ocular, renal signs. In addition, swelling of joints and lymph nodes, sudden lameness due to arthritis may be noted. Other common symptoms include lethargy, inappetence, depression and a fever that runs between 103 and 105°F. In untreated dog Lyme disease infection can cause severe progressive kidney disease known as “Glomerular disease” which frequently results in death (Carmel, 1989).

DIAGNOSIS

The diagnosis is based on history and objective clinical findings and supported by appropriately chosen laboratory test(s). Lyme disease shares many of the symptoms with the other diseases therefore there are a fair chances that someone may misdiagnose arthritis and neurological signs as Lyme disease. Though early stage of the infection relies on the clinical picture, in later stage serological, molecular, microbiological and histopathological findings are important (Mullegger, 2004).

The specimens skin biopsy, slit smear, intradermal aspirates, blood and CSF are collected for examination. For culture specimens are inoculated into Barber-Stonner- Kelly (BSK) medium. If neurologic involvement is present it is worth to examine cerebrospinal fluid samples for presence of moderate lymphocytic pleocytosis. Direct examination dark field microscopy and Giemsa staining can be are used to demonstrate the organisms. Once the diagnosis of chronic Lyme disease is made, patients are commonly treated for months to years with multiple antimicrobial agents (Henry *et al.*, 2007).

Serology: The CDC has recently updated the clinical and serologic criteria to standardize surveillance for Lyme disease. Four fold rise in titre of specific IgM antibodies with supportive clinical signs are to be considered positive (CDC, 2008).

ELISA: Antibodies to Bb are covalently coupled to a specific enzyme and allowed to bind to Borrelia antigens in the presence of the enzyme substrate. *B. burgdorferi* infection in humans and other animals results in production of killing (borreliacidal) antibodies (Rousselle *et al.*, 1998; Exner *et al.*, 2000). Borreliacidal antibodies can be detected in dogs one week after attachment of infected tick and can distinguish between early and late stage too (Callister *et al.*, 2000).

Fluorescent Antibody Test: A highly specific antibody against Bb is used to perform this test. Blood sample can be used for this test (Margaret *et al.*, 1996).

Western blot test: This test detects the presence of Bb protein antigens in serum under investigation. Its sensitivity, is comparable with that of ELISA and IFA. Considered as a good quality test for identifying Lyme disease in patients with late-stage symptoms such as arthritis. False positive results may be caused by cross reactions in patients with other infections (e.g. syphilis) or autoimmune diseases (e.g. rheumatoid arthritis).

Polymerase Chain Reaction (PCR): Blood, plasma, buffy coat, CSF, urine and suspected ticks can be used to diagnosis (Lebech, 2002). Detection of *Borrelia*-specific DNA by PCR in biopsy samples from skin lesions is most sensitive, specific and quickest diagnostic tool in early and late manifestations of dermatoborrelia (Brettschneider *et al.*, 1998). The organism appears to be better detectable using nested *OspA* instead of flagellin primers.

TREATMENT

Antibiotic treatment is necessary for all stages and manifestations. Oral therapy with antibiotics for early-localized disease while, intravenous antibiotics can be effectively used for early-disseminated and late stage. Oral therapy in Adults includes doxycycline and amoxicillin (Steere *et al.*, 1980, Steere *et al.*, 1983).

In antimicrobial therapy, doxycycline is relatively contraindicated in pregnant women and children of less than 8 years. Patients with neuroborreliosis treated with either oral doxycycline or parenteral therapy with cefotaxime or ceftriaxone. In pregnant women, the drugs of choice are amoxycillin, azithromycin and third generation cephalosporins in the same dose and duration as for non-pregnant ladies (Steere, 1989). In animals tetracycline, amoxicillin and penicillin are effectively used (Wormser *et al.*, 2006).

PREVENTION AND CONTROL

Firstly, for preventing disease it is better to avoid the ticks by using proper cloths, insect repelling creams, and sprays containing a 20-30% concentration of DEET on clothes and on exposed skin (Sider *et al.*, 2012). Control of tick population can be achieved by keeping dogs or cats tick free so that they cannot bring ticks in houses.

Secondly, early diagnosis and treatment with suitable antimicrobials secures good prognosis. As a prophylactic measure oral 200 mg single dose of doxycycline administered within 72 hours of tick bite can be done although some controversy remains (Nadelman *et al.*, 2001)

Vaccination: In year 1998, FDA developed a vaccine called 'LYMERix' which was intended for use in individuals of 16 years or elder. The vaccine had been reported with an efficacy approximately 70-80% after the full course of 3 doses (Steere *et*



al., 1998). The use this vaccine was proved to be cost-effective control in endemic areas for the population at risk (Steer *et al.*, 2004). However complaints started to rise regarding its side effects especially arthritis making officials to withdraw the vaccine form market in year 2002. Recently researchers are focusing on use of potential antigens for the making of advanced second generation vaccine (Wallich *et al.*, 2003).

Public Health Significance: Lyme disease is an emerging metazoonosis. It is a chronic disease of joints and nervous system. The persons at risk include pet owners, campers, hikers, veterinarians, farmers and people in outdoor occupations. Communities, living in houses built in heavily wooded areas where infected ticks are common are especially at higher risk. Lyme bacteria can be demonstrated in stillborns and infants born with severe abnormalities. The great importance relating to a toxin produced by the lyme bacteria as, *Borrelia* toxin is similar to the botulinum neurotoxin. Lyme toxin and other related substances is so great importance that bio-terrorists have long considered using them in terrorist attacks throughout the world (Bradford and Allen, 2005).

CONCLUSION

Lyme disease is an important vector-borne multisystemic disease having ability to cause persistent infection in a wide variety of mammalian hosts. Lyme disease is worldwide in distribution and has been reported from almost every country including India. The organism is maintained in nature either in the Sylvatic (wild) or domestic cycle. *Ixodes* tick is the main vector for the organism. Mammals, rodents and birds may also acts as natural reservoir, while dog and cat seems to be most common domesticated animal reservoirs. Climatic changes, as increased temperature shortens pathogen development time in vectors. This in turn increases the duration of infectiousness, allowing for prolonged periods of transmission to humans. The hematogenous spread of pathogen may lead to involvement of multiple organs systemic manifestations. Doxycycline is the antibiotic of choice for most patients with dermatoborrelia. Early detection of signs and symptoms of Lyme disease is very important for prompt diagnosis, prognosis and treatment. Disease can be controlled and prevented by personal protection, tick control and post-exposure antibiotics and increasing the awareness in public would be a successful step in the right direction to achieve this goal.

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