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Borrelia Lyme Group

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ABSTRACT

Borreliaceae is a family of the phylum Spirochaetales and includes two genera, Borrelia and Cristispira genus. Borrelia genus is divided into three groups, namely Lyme group (LG), Echidna-Reptile group (REPG) and Relapsing Fever group (RFG). All Borrelia species have an obligate parasitic lifestyle, as they depend on their hosts for most of their nutritional needs. Borreliæ are transmitted among vertebrate hosts by arthropod vectors (ticks and lice). Transtadial transmission within their carriers occurs for the Borreliæ RF Group, while this does not (or rarely occurs) for the Borreliæ Lyme Group.

Phylogenetic data demonstrated that these two groups are genetically similar but distinct, forming independent clades sharing a common ancestor. In nature, the vectors of LB belong to the genus *Ixodes* spp. frequently found in the Northern Hemisphere, while the vectors of RF are usually the soft-ticks (*Ornithodoros* spp.). Borreliae share a unique genomic structure consisting of a single highly conserved linear chromosome and several linear and circular extrachromosomal plasmids which can vary widely between strains. In addition to Lyme and RF borreliosis, an intermediate group, called Echidna-Reptile borreliosis, has recently been identified.

Lyme disease (LD) is caused by the spirochæte *Borrelia burgdorferi* sensu lato (s.l.) and transmitted to humans by the bite of a hard tick of the genus Ixodes, and LD reservoir are usually small rodents. LD is present in America, Eurasia, Africa, while its presence in Australia is not yet well documented.

Not all Borreliæ Lyme Groups cause this disease in humans. Of the 23 *Borreliæ burgdorferi* s.l. currently known only 9 have been identified in human infection, namely *Borrelia burgdorferi* sensu stricto, *B. afzelii, B. bavarensis, B. bissettii, B. garinii, B. lusitaniae, B. spielmani, B. valaisiana*, and *B. mayonii*. LD is an organotropic infection, but there is also a spirochætemic form, caused by *Borrelia mayonii*, which gives fever similarly to the Borreliosis RF Group. A third variant of LD is Baggio-Yoshinari Syndrome (BYS), which is transmitted by another hard tick, *Amblyomma cajennense*. This Borrelia has not been isolated in culture, therefore its membership in the Lyme Group is not yet proven. All three of these Sub-Groups can manifest early with erythema migrans. Clinical features of LD are wide and variable, with clinical manifestations linked to distinct tissue tropisms of specific *Borrelia burgdorferi* s.l. genospecies. The early infection is localized and, in the absence of treatment, the spirochete can spread. The organs most frequently involved are skin, joints, muscles, nervous system, heart and eyes. *B. burgdorferi* s.s. is more often associated with Lyme arthritis, *Borrelia garinii* with neuroborreliosis and *B. afzelii* with acrodermatitis chronica atrophicans.

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Introduction

Lyme borreliosis (BL) is a multisystemic bacterial anthropozoonosis which in humans is usually organotropic, rarely spirochætemic, and can affect the joints, nervous system, skin, heart and eyes [1]. The clinical picture is sometimes, in its late manifestations, complex and simulating, as also in syphilis, several cutaneous and neurological diseases, with which the differential diagnoses must be done, for this reason it is called the "Great Imitator".

Borreliæ belong to the Spirochaetaceae family, and have the characteristic spiral shape. Spirochaetae cause several important diseases in humans, including Syphilis, Leptospirosis and Lyme Borreliosis (LB).

The subdivision of the spirochetes is based on phylogenetic analyses of chained sequences, and the identification of INDEL sequences, including 38 specific to all members of Phylum Spirochaetes, and 16 conserved signature organism (CSI)specific to the genus Borrelia [2].

Table 1: Phylum Spirochaetes

CLASS	ORDER	FAMILY	GENUS	SPECIES/GROUP
SPIROCHÆTÆ	Brachispirales	Brachyspiraceae	*Brachispira spp*	
	Brevinematales	Brevinemataceae	*Brevinema spp*	
	Leptospirales	Leptospiraceae	*Leptospira* *Leptonema spp* *Turneriella spp*	*Leptospira interrogans*[1]
	Spirochaetales	Spirochaetaceae	*Marispirochaeta spp* *Spirochaeta spp* *Treponema*	*Treponema pallidum*[1]
		Borreliaceae	*Cristispira*	Cristispira pectinis[2]
			Borrelia spp	LYME GROUP
				Echidna-Reptile Group
				Relapsing Fever Group

[1]Spirochætæ which cause infections in humans.

[2] Cristispira pectinis was isolated from the oyster Crassostrea virginica. It could not be cultured in vitro, and the 16S rRNA genes were directly amplified by bacterial DNA; it does not cause infections in humans [3]. Sequence comparisons of the gene encoding the 16S rRNA (rDNA) insert of a clone, designated CP1, indicate that they belong to the spirochætales Order [4].

Borreliæ are divided into three main groups. The current taxonomic view is connotative of accurate evolutionary relationships and is based on:

«Conserved Signature Insertions/deletions» (CSIs) and

«Conserved Signature Proteins» (CSPs).

The distribution of a CSI is indicative of shared ancestry within the clade for which it is specific. In this way the distributions of different CSIs provides the means to identify different orders and families within the phylum and thus justify the phylogenetic divisions.

- Borrelia Lyme Group
- Borrelia Echidna-Reptile Group
- Borrelia Relapsing Fever Group

Phylogenetic relationship of 23 Borrelia genomes depends on sequence conservation [5]. The phylogenetic tree was inferred using maximum likelihood depends on sequence conservation. analysis of a concatenated alignment of 590 single copy orthologous genes. It has not yet been possible to cultivate several Borreliæ, including those of Baggio-Yoshinari Syndrome, making their classification still uncertain [6].

Ecology

The vector is a hard tick, a carrier, which can become infected with a vertebrate, and then transmit the bacterium to another. It does not contain the bacteria in a passive way, as the latter is able to reproduce within the vector itself. Ticks develop in three stages: larva (hexapod), nymph and adult (octopods). *Borrelia burgdorferi* does not normally have vertical transmission in *Ixodes* spp. ticks, except in rare cases [7].

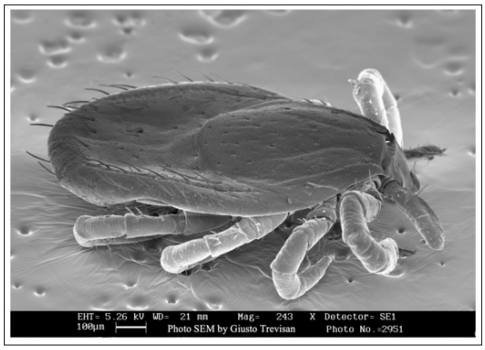


Figure 1: Hard tick nymph *Ixodes ricinus*

Humans, who thus get sick, can be considered occasional hosts. The aggressive capacity of the tick towards humans has been known in Italy since 1888, when Antonio Berlese Italian Entomologist (Padua, June 26, 1863 - Florence, October 24, 1927) wrote: «*habitat in bobus, capris, capreolis, ovibus subinde etiam hominibus infestus*». These ticks belong to the genus *Ixodes* spp.

In the United States on the East coast and in Canada the most frequent vectors of LB are black ticks, *Ixodes scapularis*. *Ixodes pacificus* is prevalent on the West coast of the US and *Ixodes affinis* in North Carolina [8].

In Europe and Eurasia the main vector is *Ixodes ricinus*, which is widespread in both cold and temperate areas and is found from the southernmost regions of Sweden and Finland to regions of the Central-Western and Southern Europe up to the North-Western Coasts of Africa (Tunisia, Algeria, Morocco). In Italy *Ixodes ricinus* has been reported above all in the Northern and Central regions, where there are humid forest biotopes, and its frequency progressively decreases from subalpine to Apennine and more Southern areas, where it is often replaced by another species frequently confused with it, *Ixodes gibbosus*. Due to the drier climate it is widespread in Abruzzo and Sardinia [9-11].

The wide diffusion of *Ixodes ricinus* is due to its high ecological plasticity, therefore it can spread in environmental situations even very different from its optimal habitat. *I. ricinus* (Figure. 1) is originally the sheep tick, but it can parasitize many mammals, being an endo-exophilic species with low parasitic specificity, therefore it can infest many different animals, including humans. In Asia and Eastern Europe (Russia) the main vector is *Ixodes persulcatus*.

LB is less common in the Southern Hemisphere and has different features. In Brazil, Baggio-Yoshinari Syndrome has been described. The syndrome is characterized by erythema migrans and *Borrelia* is here transmitted by another hard tick, more often *Amblyomma cajennense* [12]. Borrelia has not yet been isolated in culture from Baggio-Yoshinari Syndrome patients but it was identified by molecular biology techniques, therefore its precise location requires further information.

Table 2: Main Ticks and Borrelia Lyme Group in Northern and Southern Hemispheres

NORTHERN HEMISPHERE: MAIN VECTORS OF BORRELIÆ LYME GROUP		
Ixodes species	**Geographic Area**	***Borrelia burgdorferi sensu lato***
Ixodes ricinus	North Central Western South Europe, Turkey	*Borrelia afzelii, B. burgdorferi ss, B. garinii, B. lusitaniae, B. spielmani, B. bavarensis, B. valaisiana.*
	North-Africa	*Borrelia lusitaniae*
I. frontalis	Germany, Switzerland	*Borrelia burgdorferi sl*
I. scapularis	Eastern Central USA Canada	*Borrelia burgdorferi sensu stricto, B bissettii, B. carolinensis, B. kurtenbachii, B. mayonii*
I. pacificus	Western USA and Canada	*Borrelia burgdorferi ss, B. carolinensis, B. lanei*
I. spinipalpis	Western USA, Canada, Mexico	*Borrelia lanei*
I. dentatus	Northeast USA, Canada	*Borrelia burgdorferi ss, B. andersoni*
Ixodes uriae	North and South Atlantic	*B. garinii*
I. persulcatus	Russia, Eastern Europe	*Borrelia garinii B afzelii, B bavariensis*
	Northwest China, Korea, Japan	*Borrelia garinii (Asian strain NT 29), B afzelii*
I. granulosus	Central China Yangtze River Valley, Malaysia, Nepal	*Borrelia sinica B. valaisiana, B yangtzensis*
I. tanuki	Japan	*Borrelia tanuki*
I. turdus	Japan	*Borrelia turdae*
SOUTHERN HEMISPHERE: MAIN VECTORS OF BORRELIÆ LYME GROUP		
Amblyomma cajennense	Brazil Amazon rainforest	*Borrelia burgdorferi ss (?)*
Ixodes longiscatus	Brazil, Pampa Argentina	*Borrelia burgdorferi*
Ixodes paranaensis	Brazil	*Cand. Borrelia ibitipoquensis [13]*
I. affinis	Argentina, Columbia, Panama, Venezuela	*Borrelia burgdorferi sl*
I. kingi	Mexico	*Borrelia burgdorferi sl*
I. aragaoi	Uruguay	*Borrelia bissettii and B. americana.*
I. pararicinus	Uruguay Argentina	*Borrelia bissettii B. burgdorferi sl*
I. stilesi	Chile	*Borrelia chilensis*

Reservoirs

Reservoirs, or reservoir hosts, are animals, which maintain the circulation of spirochetes in nature. They have a lot of spirochetes in their blood, the ticks that feed on these animals become infected and the spirochetes multiply, spread throughout the body and persist there for a considerable period [14]. These animals are usually small rodents (mice, voles and squirrels), birds, (both stationary and migratory), reptiles and insectivores. In Switzerland, 6-18% of migratory birds were found to be infested with ticks. The ticks were *Ixodes frontalis* and *Ixodes ricinus*, containing *Borrelia valaisiana*, *B. garinii* and *B. lusitaniae*. The frequent presence of *Borrelia lusitaniae* in *Ixodes ricinus* larvae suggests the possibility that migratory birds may be reservoirs of this Borrelia [15]. Migratory birds, in particular passerines, in addition to being possible reservoirs of the Borreliæ Lyme Group, travel for even thousands of kilometers, therefore have the ability to carry the infection to other distant geographic areas, actively participating in the spread of the disease.

Other hosts, such as humans, and other mammals have low blood circulation of spirochetes, and do not transmit Borrelia Lyme Group to ticks that feed on it [16].

Table 3: Main *Borrelia burgdorferi* sensu lato reservoirs

MAIN RESERVOIRS		RESERVOIRS	
Peromiscus leucopus	North-America	*Tamias striatus*	North-America
Apodemus flavicollis	Europe	*Tamias sibericus*	Russia France
Apodemus sylvaticus	Europe	*Eliomys quercinus*	Europe
Apodemus speciosus	Asia	*Phasianus colchicus*	Europa, Black Sea
Apodemus ainu	Japan	*Streptoprocne biscutata*	Brazil
Niviventer confucianus	China	*Fratercula arctica*	North Atlantic
Myodes glareolus	North-Europe	*Turdus merula*	Europa, North-Africa, China
Sciurus griseus	North-America	*Melospiza melodia*	West USA, Pacific Coast
Sciurus carolinensis	North-America UK	*Geothlypis trichas*	Canada, USA, Mexico, Pacific Ocean
Tamiasciurus hudsonicus	Minnesota Wisconsin		

Epidemiology

The distribution of Lyme Borreliosis and tick-borne diseases is mainly related to the tick density and the presence of animals [17]. Climate change is influencing worldwide the increment of vector-borne diseases, including Lyme disease and tick-borne encephalitis, particularly in North America and Europe [18]. The geographic distribution of LB is indeed related to the distribution of *Ixodes* vectors and to climate change. The climatic conditions limit the latitudes and altitudes in the distribution of the ticks. Although detection by molecular or immunological methods has improved, tick-borne diseases continue to remain underdiagnosed, making difficult to assess the extent of the problem.

Lyme borreliosis is prevalent mainly in the Northern hemisphere. In North America, 90% of cases are reported mainly from two regions of the USA: the North-Eastern and Mid-Atlantic region and the North-central region.. Both regions have expanded substantially over the past 20 years and have reached the Southern areas of Canada [19].

In US, tick species that bite humans transmit collectively more than 15 species of pathogenic microorganisms and the national burden of tick-borne diseases is increasing. The estimated annual cases of Lyme disease are 450,000. The main vector of Lyme disease in the eastern United States is *Ixodes scapularis*, which can also transmit other tick-borne diseases, such as anaplasmosis, babesiosis, and Powassan encephalitis [20]. In Kentucky, where LB incidence was low, cases are increasingly growing [21]. In Canada the marmot tick (*Ixodes cookei*), also widespread, is a vector for *Borrelia burgdorferi* sl and can also bite humans [22].

In Europe there have been reported 85.000 new cases/year and 360.000 new cases estimated. Most reported cases have been diagnosed in temperate regions and have been underestimated [23]. The incidence is indeed highly variable in different countries, probably due to the different notification methods. The country where the incidence appears highest is Sweden with 632 cases / 100,000 inhabitants / year [24]. Cases of LB have been reported in Austria, Belgium, Bosnia, Bulgaria, Croatia, Czech Republic, Denmark, Estonia, Finland, France, Germany, Greece, Hungary, Iceland, Ireland, Italy, Norway, Russia, Sweden and the United Kingdom. In endemic areas these ticks are infected with Lyme Borrelia in 20-40% of cases [25]. In Southern Europe (Italy) and the Northern countries (Sweden) *Borrelia afzelii* is prevalent, while in Central Europe *Borrelia garinii. Borrelia burgdorferi* ss is found in Western Europe, as it is usually not present in *Ixodes persulcatus* (Eastern Europe) [26]. In the Western Siberian region *Borrelia afzelii* and *B. garinii* have been identified in *Ixodes persulcatus*, while *Borrelia bavariensis* was isolated from *Ixodes persulcatus* and *Borerelia garinii* from *Ixodes pavlovskyi* [27, 28]. In Turkey up to 2019, 75 cases of LB have been described, and the studies related to LB are case reports, therefore its real incidence is likely to be underestimated [29].

LB is present in most Asian countries [30]. In Southeast Asia (Japan, Northeast China and Mongolia), *Borrelia garinii* and *B. afzelii* have been isolated from *Ixodes persulcatus* [31]. *B. burgdorferi* sl has also been isolated from other *Ixodes spp* ticks, which fed on migratory birds therefore can spread LB in Asian continent [32]. In China *Borrelia sinica* has been isolated from *Ixodes granulatus* (unknown to cause disease in humans), and *Borrelia yangtzensis* from ticks and small rodents [33].

In this country inn 25 provinces over the period 1986-2020, a total of 2,584 confirmed human cases was reported. *Borrelia burgdorferi* was detected in 35 tick species with the highest rates in *Ixodes granulatus* [34]. In Japan *B. valaisiana* has been isolated from *I. columnae* [35]. Other Lyme Group Borreliæ, such as *B. japonica* from *I. ovatus*, B. *tanukii* from *I. tanuki*, *B.turdii* from *I. turdus* are not pathogenic to humans. In Korea, *Borrelia valaisiana* and B. *garini* from *I. nipponensis* have been identified [36]. LB could be also transmitted in India by *Ixodes acutitarsus*, *I. himalayensis*, *I. kashmericus*, where cases of erythema migrans with positive serology and cases of neuroborreliosis have been reported. *Borrelia yangtzensis* was also found in *Ixodes granulatus* in Malaysia [37-39].

In North Africa (Tunisia, Morocco, Algeria) *Borrelia lusitaniae*, the vector of which is *I. ricinus* is the prevalent species [40]. Two cases of LB characterized mostly by neurological manifestations have also been described in Kenya [41].

Rare cases of *Borrelia* genome-positive erythema migrans have been reported in Australia, but the isolation in culture of Lyme group Borrelia has not yet been possible [42].

Microbiology

Morphological and Metabolic Characteristics

At the transmission electron microscope (TEM), Borreliæ have the typical spiral shape shared with all spirochetes, that have also thin bacterial body, endo-flagella and are extremely labile to environmental factors. This lability comes from the poor biosynthetic activity of the microorganism which is dependent from environments rich substrates (host, soils). The protoplasmic cylinder is surrounded by an outer membrane [43], similar to that of Gram negative bacteria. It is rich in lipoproteins, which are differentially expressed in the mammalian host and in the vector, but is lacking of lipopolysaccharides, causing little protection against external environmental agents, therefore inducing host parasitism. The musculoskeletal system is composed of flagella that twist along the entire length of the bacterial body and are included between peptidoglycan and the external membrane (figure 1). This structure allows moving effectively through viscous media, to pass through capillaries and invade the connective

tissue, penetrating deeply into tissues. Peptidoglycan contains L-ornithine as a distinctive element.

Table 4: Distinctive morphological characteristics of Borrelia burgdorferi now belonging to the Borrelia Lyme Group

Microaerophilic	
Shape	Spiral
Length	7-24 µ
Width	0.20-0.50 µm
Propeller pitch	1.7-3.3 µm
Number of flagella	7-12
Peri-flagellar sheath	Absent
Outer Surface Membrane	Three-layers. It envelops a periplasmic space, containing the flagella and the protoplasmic cylinder
Cytoplasmatic tubules	Absent

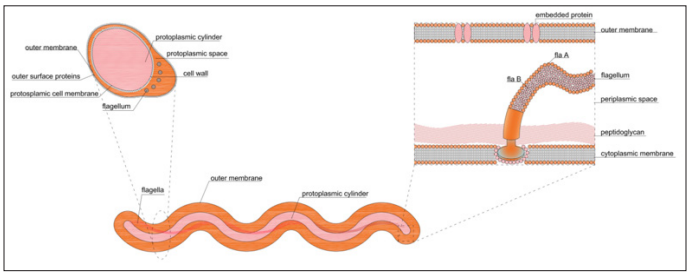


Figure 2: Borrelia Lyme Group Scheme

Metabolism and Culture

The metabolism derived from the *in vitro* cultivation of the microorganism is microaerophilic. In culture, the doubling time of the bacterium is 16 hours. It is superoxide dismutase positive, catalase and peroxidase negative. *Borrelia burgdorferi* sensu lato does not grow in normal bacteriological media, but needs complex nutritional factors, provided by the medium Base CMRL-1066 (culture medium for cell cultures containing all amino acids, and vitamins) rabbit serum, with the addition of N-acetyl-glucosamine and peptoglycan growth factor [44]. The final optimal formulation, BSK II medium (Barbour-Stönner-Kelly II) was developed by Barbour. However, a slightly different composition by Slovenian authors, the MKS 2 medium (Kelly-Pettenkofer), seems to be more efficient in the isolation of t strains from patients tissues and seems also particularly suitable to cultivate *Borrelia miyamotoi*, which belongs to the Hard-Tick-Borne Relapsing Fever (HTBRF) Borreliæ, but it is transmitted by the same Ixodes spp ticks, of the Lyme Group Borreliæ. Another variant is the Barbour-Stönner-Kelly-H (BSK-H) medium. It is also possible to cultivate *B. Burgdorferi* in solid medium, obtained by adding a solidifying agents, such as agarose. Incubation is carried out at 33-34 ° C in humidified chambers in order to prevent the soil to dry out. After at least 7 days, semi-transparent colonies rich in borrelia can develop. This technique allows cloning the first isolation strains, often mixed, but does not guarantee a reliable vital count.

The first isolation was carried out in 1982 by Willy Burgdorfer and Alan Barbour from *Ixodes dammini* (*Ixodes scapularis*) tick, identifying *Borrelia burgdorferi* sensu stricto, strain B31 [45]. In Italy the first isolation in culture was carried out in 1997 by Marina Cinco from *Ixodes ricinus* tick with the identification of *Borrelia garinii* strain BITS (Borrelia Italy Trieste) [29].

The Borrelia Burgdorferi Genome

The genome of B.b. has a peculiar organization, which appears singular in the context of prokaryotes. It is indeed divided into a linear chromosome of about 1000 kilobases and a lot of plasmids, some of which are linear and others circular. One of these, the linear 49 Kb plasmid, encodes the immune-dominant surface proteins OspA and OspB. Other smaller circular plasmids of 8 Kb are related to the virulence of *Borrelia burgdorferi* and are lost during *in vitro* cultivation. A total of 17 plasmids have been listed, some of which encode characters related to the pathogenicity of B.b. and some are related to the antigenic variability of Borrelia, thus to immune evasion *in vivo*.

B. burgdorferi chromosome has been sequenced [46]. It contains 853 genes encoding a set of basic proteins for DNA replication, transcription, translation, solute transport and energy metabolism, but similarly to *Mycoplasma genitalium* it does not contain genes for biosynthetic reactions. This would explain the huge demands of these spirochetes for pre-formed nutrients. *B.burgdorferi* chromosome contains coding sequences for 16S rRNA (rrs) separated by pair of genes for the 23S ribosomal subunit (rlAe rlB) and the 5S ribosomal subunit (rfA and ttfB). This peculiar organization of genes for rRNA represents a molecular target for genotyping, which can be carried out using the following methods:

- 16S-23S ribosomal RNA spacer (IGS) and Outer surface protein C gene (OspC) sequences;
- Multy-locus sequence typing (MLST), by the use of DNA sequences at multiple housekeeping loci characterizing genetic variations of *Borrelia burgdorferi* sl;
- Molecular Phylogeny.

Borrelia Burgdorferi Sensu Lato Antigens and Proteins

Flagellin is a genus-specific protein of the flagellum, with a molecular weight of 41 KDa, and has some homologies with other spirochetes (*Borrelia hermsii, Treponema pallidum, Treponema phagedenis*) inducing possible serological cross reactions. Flagellin gene is highly conserved, but differs in sequence between Borreliæ Lyme Group species. Tryptic cleavage of the recombinant flagellin of B. burgdorferi sensu lato expressed in *E. coli* produced a peptide fragment that was recognized exclusively by the antisera of the Borrelia species. This peptide has a mass of 14 kDa (strain *Borrelia burgdorferi* sensu stricto GeHo and strain *B. afzelii* PKo) [47]. The p14 flagellin peptide was used as antigen in ELISA and Western-Blot, demonstrating a greater specificity in comparison to intact flagellin [48].

Outer surface proteins (Osps)- In the outer membrane of Lyme Group Borreliæ the outer surface proteins, lipoproteins anchored to the surface, are localized [49]. Some of these are immunodominant and can be expressed or derepressed during the biological cycle in a different way in the vector and in the mammalian hosts (including humans). Since the pathogen is extracellular, the surface Osp proteins play a role as virulence factors by mediating adhesion to the host tissues or evading the immune system, through antigenic variations, complement inhibition and / or the formation of immune complexes.

Osp A and Osp B are lipoproteins of the outer membrane of *Borrelia burgdorferi* sensu lato. OspA has a molecular weight of 30-33 KDa and Osp B 34 KDa. They are encoded by genes located on a single linear plasmid of 54 Kb in a single transcriptional unit. They are expressed by *Borrelia burgdorferi* sl in large amounts when Borrelia is in the tick, where they allow the microorganisms adhering to the walls of the intestine, while they are not expressed in mammals and humans (or can be expressed in the late phase

of the disease). In the transmission to the mammalian host, when the nymphal tick begins to feed on blood, the spirochetes in the intestine of the tick multiply rapidly and are transferred from the vector's environment at 20-25 ° C to one (e.g. man) at 35 ° -37°C [50]. This increment of temperature seems to be responsible that most Lyme Group Borreliæ stop expressing OspA and OspB on the surface and express OspC, which is activated during the first day of the blood meal and peaks 48 hours after the *Ixodes* spp. tick bite [51].

OspC is encoded by sequences of the 27 Kb circular plasmid and is a complex of lipoproteins of 21-25 kDa located on the surface of the external membrane. OspC during early human infection (erythema migrans) allows the adhesion to fibronectin and dermatan sulfate (components of collagen in mammals) and induces an early immune response in IgM (early antigen). This response is highly specific, and anti-OspC antibodies are therefore a marker of infection.

OspD gene is located on a 38 Kb linear plasmid and encodes a protein of 28 KDa, which is usually expressed *in vitro* after about 7-9 passages in culture. It is under-expressed in mammals and over-expressed in the tick. OspD is not essential for the infectious cycle of Lyme Group Borreliæ, but plays an important role during bacterial replication within the gut of the tick [52].

OspE and OspF (Erps) are surface lipoproteins of 19 and 26.1 KDa respectively; they are encoded by a polycistronic operon located on a 45 Kb plasmid. OspE and OspF genes are arranged into tandem in the form of a single transcriptional unit, under the control of a common promoter. The proteins are expressed in the mammalian host and are related to the possibility of adhering to the host's tissues. OspE is able to bind the FH factor, regulator of the alternative complement pathway as well as plasminogen and OspF acts in particular as a powerful adhesin for the myocardium [53]. The *Extracellular Matrix Ligands* DbpA and DbpB bind the decorines, which are fundamental components of the connective tissue. They are late antigens important in the dissemination of spirochæte and allow the localization in collagen-rich tissues [54]. The p47lipoprotein is expressed by the BBK322 gene and binds fibronectin, acting as a true adhesin and with DbpA it is important in the attachment to the connective matrix.

Heat Shock Proteins (HSP) are proteins located under the surface. They are antigenically cross-reactive with other pathogens, such as Legionella, Listeria, Mycobacterium, Pseudomonas, Salmonella. Their sequence is highly conserved and can also be found in the mitochondria of prokaryotes. Five to seven HSPs were detected in *Borrelia burgdorferi* sl by SDS PAGE electrophoresis [55].

VlsE (*Variable major protein-like sequence Expressed*) has a mass of 35 KDa. It consists of a variable external part and an internal, preserved part. The locus VlsE has 15 silent cassettes, where 6 variable and 6 preserved portions are present. The variables ones are exposed on the surface and change the antigenic structure during the infection, thus evading the antibodies, while the internal portion is used in diagnostics, where it represents an important early antigen [56]. For serology, all the conserved antigen (VlsE) or the sixth portion, the most immunogenic indicated by the term IR, or C6 when it is produced synthetically, can be used.

Figure 2 shows the 23 Lyme Group Borreliæ known at present. They are divided into those that cause infection in humans (*Borrelia burgdorferi ss, B. garini, B. bavarensis, B. afzelii, B. valaisiana, B. lusitaniae, B. spielmani, B. bissettii, B. carolinensis, B. mayonii*), and those that are unknown to cause human infection. *Borrelia americana* and *B. kurtenbachii* seem to have caused Lyme in humans in a few cases, but this has to be confirmed.

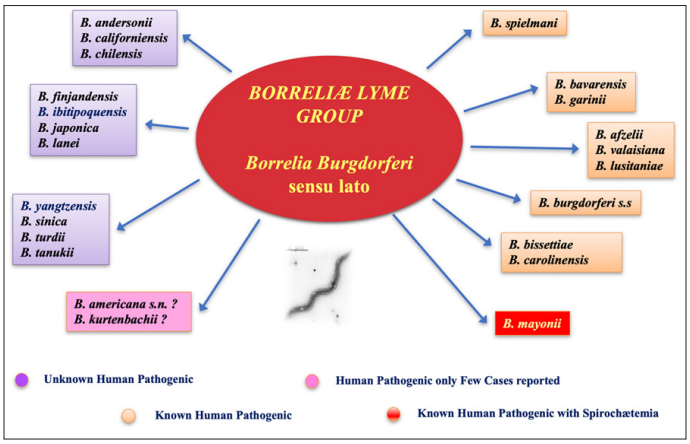
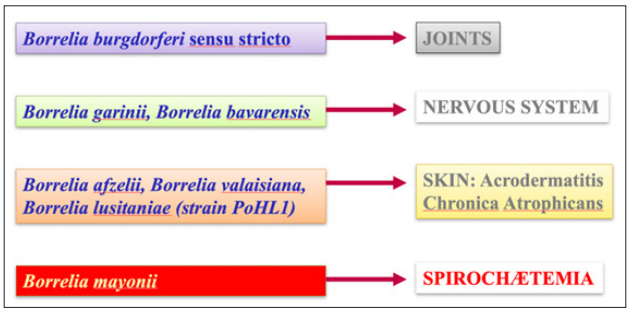


Figure 3: Borrelia burgdorferi sensu lato heterogeneity

Characteristics and subdivision of the Lyme Group Borreliæ

Lyme Group Borreliæ, which cause infection in humans, are in most cases organotropic and have often a preferential tropism. There is indeed a correlation between the clinical manifestation of LB and the Borrelia genospecies (Figure. 4). *B. burgdorferi* ss causes mainly arthritis; *B.garinii* is usually associated with neuroborreliosis. The extent of infection of this genospecies, which is transmitted by various species of Ixodidae, is singular: it can find its reservoirs not only in small mammals, but also in migratory birds (e.g.: *Ixodes uriae* ticks of seabirds) [57]. *B. afzelii* is associated to skin lesions such as Acrodermatitis chronica atrophicans, a typical late "European" skin manifestation of LB. *B.mayonii*, recently isolated from febrile patients in the Mid-Western United States, unlike the other Lyme Group Borreliæ, is able to give spirochætemia, reaching a bacterial load of 106 microorganisms / ml. Using an eight-gene multi-locus sequencing (MLSA) assay, the spirochete was identified as a new *B. burgdorferi* sl genospecies, and referred to as *Borrelia mayonii* [58]. It was isolated in modified BSK medium and its invasive capacity has been correlated with the presence of Complement-Regulator Acquiring Surface Proteins (CRASPs) CspA, CspZ and OspE able of neutralizing the lytic activity of the complement [59, 60].



In Lyme Group Borreliae also the ones responsible of the Brazilian Lyme-Disease-Like Illness, named «BAGGIO-YOSHINARI SYNDROME» (BYS) are included. The associated disease is present in Brazil and in the Amazon rainforest and in 50% of patients it develops initially with erythema migrans. This form differs from LB for the vector which is the hard tick *Amblyomma cajennense,* but also for distinctive clinical aspects [61]. We can therefore divide the Borreliæ Lyme Group into three subgroups:

Table 5: Lyme Sub Groups

GROUPS	SUB-GROUPS	HUMANS	CLINICAL ASPECT		HOSTS RESERVOIRS	VECTORS/ TICKS
			Erythema migrans	Fever		*Hard-Ticks*
LYME GROUP	Organotropism	Yes	Yes	No	Rodents Birds	*Ixodes sp*
	High Spirochaetemia	Yes	Yes	Yes	Rodents	*Ixodes sp*
	Baggio-Yoshinari	Yes	Yes	Yes	Unknown	*Amblyomma sp*

Distinctive Clinical Aspects in the Three Subgroups

- o Erythema migrans (all Lyme Sub-Groups)
- o Fever with Spirochaetemia (*Borrelia mayonii*)
- o Erythema nodosum (Brazilian Lyme-Disease-Like Illness - BYS)

Clinic

Lyme Borreliosis (BL) is a multisystemic infection transmitted by hard-ticks *Ixodes spp*. It is caused by the spirochæta *Borrelia burgdorferi* sensu lato (Lyme Group). The typical manifestation is erythema (chronicum) migrans (ECM). The clinical picture is sometimes complex, simulating various cutaneous and neurological diseases ("Great Imitator"). Elements characterizing and distinctive of the three subgroups of Lyme Group Borreliae are:

- Hard tick bite: *Ixodes* spp in Northern Hemisphere and *Amblyomma cajennense* in Brazilian Lyme-Disease-Like Illness - Baggio-Yoshinary Syndrome (BYS);
- Erythema migrans, which is present in all three Lyme subgroups;
- Fever with spirochætemia, only for *Borrelia mayonii*
- Erythema nodosum, in one third of cases of BYS

Clinical Phases

Lyme disease develops in three stages:

- Localized early phase or 1st stage
- Early disseminated phase or 2nd stage
- Late phase or 3rd stage

Localized Early Phase Or 1st Stage

Afzelius-Lipschutz's erythema (chronicum) migrans (EM) is pathognomonic of Lyme disease and its recognition is sufficient for the diagnosis of Lyme disease. It is the only situation where the diagnosis does not require laboratory tests [62]. EM is the first manifestation of Lyme Borreliosis, and is an emerging problem [63]. It consists of a circular erythema that develops around the site of the tick bite within 5 to 30 days from the bite, it gradually enlarges and after a few months it can reach the size of 50 cm or more. Erythema migrans is an early, but not always localized form [64].

A positive history of tick bite is useful for diagnosis. Nevertheless, the tick bite, is usually recalled in two thirds of patients, therefore a negative history of tick bite does not exclude the diagnosis of erythema migrans. It is therefore useful to know the residence or stay in an endemic area.

Erythema migrans appearance is not immediate, but after an incubation of 4-30 days from the bite. The extension of the erythematous circle around the tick bite, and the diameter usually greater than 5 cm are considered diagnostic.

The extension of the erythematous circle around the tick bite, and the diameter usually greater than 5 cm are considered diagnostic. EM can appear anywhere on the body regardless the age and gender. The most common sites are the legs and back, as well as the head in children and also the involvement of the genitals is sometime observed. In some cases, conjunctivitis (follicular) may be observed. Where EM is atypical, for example it has purpuric aspects, where there is fever and / or headache, it is advisable to investigate on co-infections (Anaplasma, Rickettsia, Babesia).

In children, head and neck region is often affected (Figure 1). For this reason, facial paralysis (Bell's) is more frequent in children, involving nearby structures and tissues.

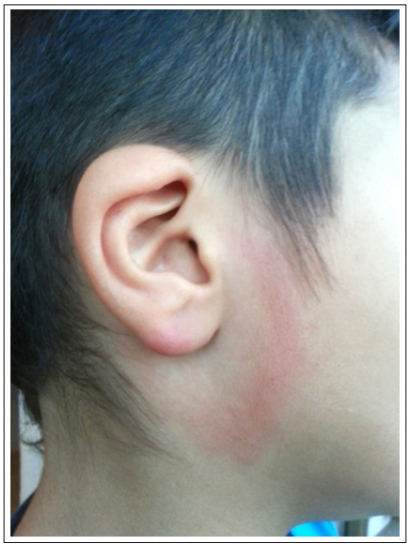


Figure 4: Erythema migrans of the face in a child

Anti-Borrelia antibodies in the EM phase can be negative, as in the first case (1992) where *Borrelia afzelii* was isolated in BSK medium from a biopsy fragment of a EM of the leg [65].

Early Disseminated Phase or 2nd Stage

When EM is not treated quickly, clinical manifestations of the disseminated form may appear.

At the cutaneous level the following manifestations can be observed:

- Multiple annular erythema
- Roseolar lesions
- Borrelia lymphocytoma

Multiple annular erythema is a sign of the early spread of the infection. Its appearance indicates the transition from the early-localized to the disseminated phase of LB. It is characterized by the appearance of circular erythematous elements, which are not centered by the tick bite mark. Several or numerous elements and can be localized throughout the skin.

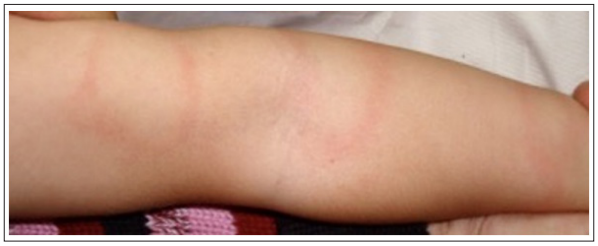


Figure 5: Multiple annular erythema of the upper limb

Roseolic erythema is a rarely observed manifestation, but in dermatology it is important to report it, as it recalls roseola due to another spirochetosis, syphilis. In one case *Borrelia garinii* was isolated from the roseolar lesion biopsy in BSK medium [66]. In Figure 6, the presence of roseolic erythema in association with the ECM is documented.

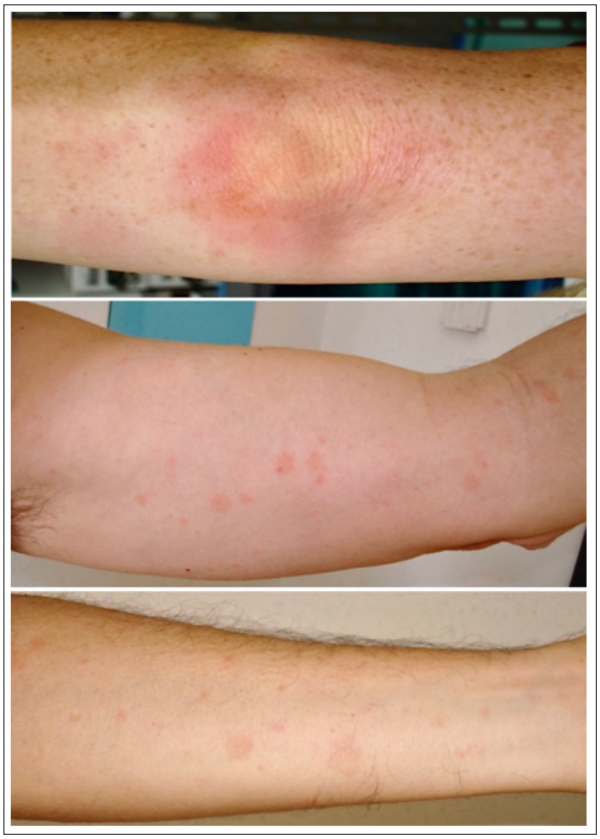


Figure 6: Erythema migrans at the elbow can be seen in the first image, and roseolar lesions on the same limb in the other two

Borrelia lymphocytoma is a B-cell pseudo-lymphoma which is due to *Borrelia burgdorferi* sensu lato antigens in the skin. Borrelia lymphocytoma is an infrequent, but typical manifestation of LB and develops weeks or months after the tick bite. It is found mainly in Europe and it is rarer in the United States, because it is usually caused by *Borrelia afzelii* and rarely by *B. garinii* or *B. bissettii* [67]. It is a subacute papulo-nodular lesion usually solitary and can be localized to the ear lobe (more often in children), to the mammary areola and less frequently to the scrotum or axillary site [68]. It may be accompanied or preceded by erythema migrans. The biopsy allows performing histological examination, immunohisochemistry for the differential diagnosis with Primary Cutaneous B- cell Marginal Zone Lymphoma (PCMZL) and PCR analysis for Borrelia Lyme Group detection.

It is therefore important to carry out the immunophenotype: CD20 +, CD21 +, Bcl2- and κ / λ ratio are expressed in equivalent manner in Borrelia lymphocytoma, while PCMZL is Bcl-2+ and has altered κ / λ ratio.

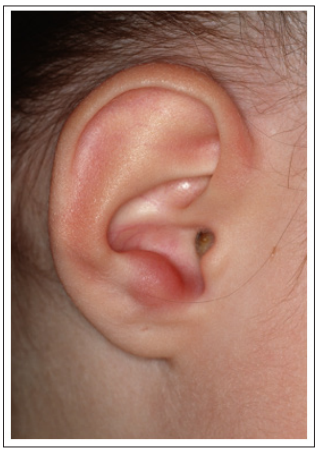


Figure 7: Borrelia lymphocytoma of the right ear

The Primary cutaneous B cell Marginal Zone Lymphoma (PCMZL) is an extranodal marginal zone (skin) low-grade B-cell lymphoma. Goodlad and Cerroni in two different articles highlighted the presence of Bb DNA in different types of PCMZL, suggesting a possible etiological role [69,70]. These studies show that in about 20% of PCMZL Borrelia DNA is detected. Differences between data collected in Europe, Asia and the USA may be associated with regional and strain diversity.

In the case in Figure 8: IgG Western Blot was positive for *Borrelia burgdorferi* as well as PCR amplifying both Flagellin and OspA in DNA from the involved tissue [71].

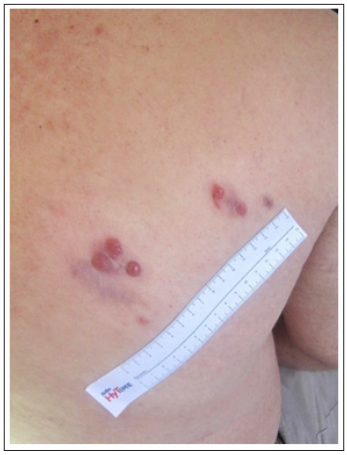


Figure 8: Primary Cutaneous B-Cell Marginal Zone Lymphoma with positive PCR for Borrelia Lyme Group in involved tissue (amplification of OspA and Flagellin sequences).

The patient, co-infected with Ehrlichia, was treated with doxycycline 200 mg / day iv for 20 days and then the nodules were treated by electrochemotherapy. A few months ago, after 8 years of treatment, the patient had a check-up and there were no signs of relapse.

Extracutaneous Manifestations

At the joint level, in the 2nd stage, mono or oligoarticular migratory arthralgias are observed. They can be localized to the large joints (scapular, pelvic, elbows, wrists, knees, ankles), and in some cases to the temporomandibular joint. Initially, the episodes are short,

of one or a few days with intervals of a couple of weeks. The first joint affected is often the one closest to the EM. As the infection progresses, the duration of myo-arthralgic episodes lengthens and intervals shorten.

Neurological manifestations (neuroborreliosis) can present with headache, (for example caused by lymphocytic meningitis, but also can be linked to other different situations), cranial nerve palsy (more frequently facial palsy), especially in children, Garin-Bujadoux- Bannwarth polymeningoradiculoneuritis, more often in adults.

Cardiac involvement can be observed in 5-10% of cases: arrhythmias (more often bradycardia), myocarditis and pericarditis.

Ocular manifestations may present with follicular conjunctivitis (even during the early localized phase), papillary edema, uveitis, keratitis [72].

Late phase (3rd Stage)

After 7-12 months, if the infection is not treated, LB evolves into the third stage.

In the skin, the typical cutaneous manifestation is Pick-Herxeimer's Acrodermatitis chronica atrophicans (ACA) (Figure 9), which usually begins at the extensor part of the of the limbs, often with an erythemato-cyanotic inflammatory phase. ACA develops several months or years after tick-borne infection and affects mainly adults, while it is rare in children [73]. Where localized to one or both hands, it simulates a form of acrocyanosis, which is an important differential diagnosis. The initial lesions progressively tend to extend, affecting the entire acral surface, and become atrophic; the skin becomes progressively smooth, thin, transparent and inelastic, showing clearly the subcutaneous vessels. Subsequently the atrophy can also involve the subcutis and the underlying muscle tissue, resulting in severe damage to the limbs [74]. The involvement of the limbs is asymmetrical and the most intensely affected limb has a smaller diameter than the contralateral [75]. In some cases, ACA is associated with patches of morphœa or lichen sclerosus et atrophicus [76]. These atrophosclerodermal forms are rare in the United States (some cases imported or patients infected in Europe) as they are related to *Borrelia afzelii*, which is absent in the US.

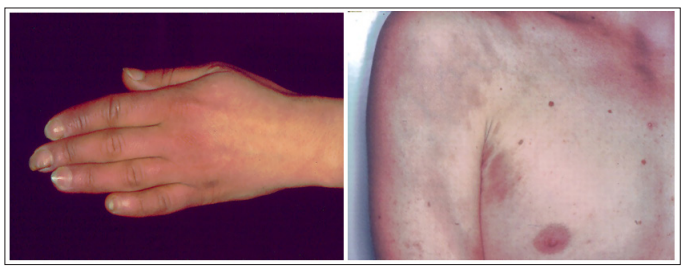


Figure 8 and 9: ACA Acrocyanotic-like lesion – ACA with *Borrelia afzelii* cultural isolation in BSK medium

Extracutaneous Manifestations

At the joints, myarthralgia progressively takes the picture of arthritis, with characteristics similar to other arthritis forms. Late neurological manifestations are not frequent, peripheral and more often secondary to ACA, due to a direct passage of *Borrelia afzelii* from the skin to the cutaneous and subcutaneous nerve fibers [77]. For this reason, in these forms the examination of the liquor cerebri is negative.

Lyme and Pregnancy

The Lyme Group Borrelia can pass the placental barrier from the first month, so it can cause miscarriage in recent infection or fetal damage. There is currently a paucity of data on tick-borne infections (LB and Co-infections) in pregnancy and on long-term outcomes for mother and baby.

Women who develop LB during pregnancy, and receive adequate antimicrobial therapy (usually Amoxicillin), do not have an increased risk of miscarriage or fetal or neonatal harm [78]. Newborns should be examined for possible clinical manifestations, as infants born to mothers with gestational LB have been documented in some cases [79].

They can be small for the date, present cardiac manifestations, pyloric stenosis, ring erythemato-papular rash, cutaneous angiomas, neurological disorders, muscle hypotonia, hypospadias and skeletal anomalies. Congenital Lyme Borreliosis is very rare. In our experience, two cases of congenital Lyme were observed. In the first one it was also possible to isolate Borrelia in BSK culture from the skin [80].

Baggio-Yoshinari Syndrome (BYS)

BYS differs from Lyme Borreliosis starting from the vector as it is transmitted by the *Amblyomma cajanense* tick, which transmits the "Spotted fever".

Erythema migrans (EM) is in 50% of patients. Headache is an important symptom for the diagnosis.

In the II stage of the disease arthritis and neuropathy are observed in 35% of cases, cardiological manifestations only in 5%. Relapsing symptoms occur in 75% of patients despite antibiotic treatment. In 30% of cases erythema nodosum is observed.

Patients have a high frequency of autoantibodies directed against the constituents of their cells: antinuclear antibodies (ANA), anticardiolipin (ACA), cytoplasmic anti-neutrophils (ANCA) and antineuronal antibodies [81].

Erythema nodosum has been observed in active Lyme Borreliosis, but it is a very rare occurrence.

It is much more frequent (in a third of cases) in BYS.

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