CONSENSUS STATEMENT OF THE SPANISH SOCIETY OF INFECTIOUS DISEASES AND CLINICAL MICROBIOLOGY (SEIMC), SPANISH SOCIETY OF NEUROLOGY (SEN), SPANISH SOCIETY OF IMMUNOLOGY (SEI), SPANISH SOCIETY OF PEDIATRIC INFECTOLOGY (SEIP), SPANISH SOCIETY OF RHEUMATOLOGY (SER), AND SPANISH ACADEMY OF DERMATOLOGY AND VENEREOLOGY (AEDV), ON THE DIAGNOSIS, TREATMENT AND PREVENTION OF LYME BORRELIOSIS

COORDINATOR

1. José A. Oteo. Centro de Rickettsiosis y Enfermedades Transmitidas por Artrópodos Vectores (CRETAV). Departamento de Enfermedades Infecciosas. Hospital Universitario San Pedro-Centro de Investigación Biomédica de La Rioja (CIBIR). C/ Piqueras, 98. 26006 Logroño (España). Phone: +34 941 298 993; E-mail: jaoteo@riojasalud.es

AUTHORS, IN ALPHABETICAL ORDER

2. Héctor Corominas, Spanish Society of Rheumatology. Rheumatology and Systemic Autoimmune Diseases Department. Hospital de la Santa Creu i Sant Pau/Hospital Dos de Maig. Barcelona (Spain).

3. Raquel Escudero, Special Pathogens Reference and Research Laboratory. Centro Nacional de Microbiología; Instituto de Salud Carlos III, Majadahonda (Spain).

4. Fernando Fariñas-Guerrero, Spanish Society of Immunology. Institute of Clinical Immunology and Infectious Diseases. YNMUN Biomedicine Group. Málaga (Spain).

5. Juan Carlos García-Moncó, Spanish Society of Neurology. Department of Neurology, Hospital Universitario Basurto, Bilbao (Spain).

6. Miguel A, Goenaga, Infectious Diseases Service. Hospital Donostia. OSI Donostialdea. Instituto BioDonostia. San Sebastián (Spain)

7. Sara Guillén, Spanish Society of Pediatric Infectology (SEIP). Department of Pediatrics. Hospital Universitario de Getafe. Madrid (Spain).

8. José M. Mascaró, Spanish Academy of Dermatology and Venereology. Department of Dermatology. Hospital Clínic de Barcelona. University of Barcelona. Barcelona (Spain).

9. Aránzazu Portillo, Center of Rickettsiosis and Arthropod-Borne Diseases. Department of Infectious Diseases. Hospital Universitario San Pedro-Centro de Investigación Biomédica de La Rioja (CIBIR). Logroño (Spain).

CORRESPONDING AUTHOR

José A. Oteo

Center of Rickettsiosis and Arthropod-Borne Diseases (CRETAV). Department of Infectious Diseases. Hospital Universitario San Pedro-Centro de Investigación Biomédica de La Rioja (CIBIR). C/ Piqueras, 98. 26006 Logroño (Spain). Phone: +34 941 298 993; jaoteo@riojasalud.es

FUNDING

No funding.

ACKNOWLEDGEMENTS:

All figures with exception of figure 8 (A,B) (provided by Dr. Mascaró from Hospital Clinic of Barcelona), have been provided by the the Center of Rickettsiosis and Artropod-Borne Diseases (CRETAV) from the Departament of Infectious Diseases of the Hospital Universitario San Pedro-Center of Biomedical Research of La Rioja (CIBIR).

ABSTRACT: The diagnosis of Lyme borreliosis (LB) is based on the epidemiological history, clinical manifestations and microbiological findings in the disseminated and late phases of the disease. Related to this fact, in recent years, microbiological diagnostic techniques have appeared. These ones, far from facilitating the diagnosis and, as such, the clinical-therapeutic management of patients suffering from LB, are creating confusion. In this consensus statement, different experts and representatives of Spanish Scientific Societies (Spanish Society of Infectious Diseases and Clinical Microbiology (SEIMC), Spanish Society of Neurology (SEN), Spanish Society of Immunology (SEI), Spanish Society of Pediatric Infectology (SEIP), Spanish Society of Rheumatology (SER), and Spanish Academy of Dermatology and Venereology (AEDV) review the epidemiology, clinical spectrum, diagnostic techniques available for the diagnosis of infection by *Borrelia burgdorferi* sensu lato, in addition to the therapeutic and prevention options of LB. In a consensual way, the recommendations to establish the correct clinical and microbiological diagnosis are offered together with the recommendations to support the therapeutic management and prophylaxis of the infection.

KEY WORDS

Lyme borreliosis, *Borrelia burgdorferi* sensu lato, Spain, guidelines.

ABBREVIATIONS

AAN: American Academy of Neurology

ACA: acrodermatitis chronica atrophicans

AEDV: Spanish Academy of Dermatology (AEDV)

AV-B: atrioventricular conduction blocks

BID: one doses every12h

BmpA: *Borrelia* membrane protein A

BSK: Barbour-Stoenner-Kelly

CDC: Centers for Disease Control and Prevention

CLIA: ChemiLuminiscence ImmunoAssay

CMV: cytomegalovirus

CNS: central nervous system

CSF: cerebrospinal fluid

DbpA: decorin-binding protein A

DEBONEL: *Dermacentor*-borne-Erythema-Necrosis-Lymphadenopaty

DEET: N,N-diethyl-meta-toluamide

ECG: electrocardiogram

EFNS: European Federation of the Neurological Societies

EIA: enzyme immunoassay

ELFA: enzyme linked fluorescent assay

ELISA: enzyme-linked immunosorbent assay

EM: erythema migrans

ESCMID: European Society of Clinical Microbiology and Infectious Diseases

HIV: human immunodeficiency virus

IDSA: Infectious Diseases Society of America

IFA: indirect immunofluorescence assay

IFA: indirect immunofluorescence assay

IR3535: ethyl-3-(N-n-butyl-N-acetyl) aminopropionate

KPM: Kelly-Pettenkofer medium

LA: Lyme arthritis

LB: Lyme borreliosis

MMIA: Multiplexed Microbead ImmunoAssay

N: number of subjects

ND: not determined

NK: natural killer

OD: one doses every 24h

OLE: oil of lemon eucalyptus

Osp: outer surface protein

PCR: polymerase chain reaction

PMD: p-menthane-3,8-diol

PNS: peripherial nervous system

PTLS: post-treatment Lyme syndrome

s.l.: sensu lato

s.s.: sensu stricto

SEI: Spanish Society of Immunology

SEIP: Spanish Society of Pediatric Infectology

SEN: Spanish Society of Neurology (SEN)

SER: Spanish Society of Rheumatology

SI: stimulation index

TBD: tick-borne disease

TID: one doses every 8h

VlsE: variable lipoprotein surface-exposed protein

WB: western blot

WHO: World Health Organization

1. INTRODUCTION AND JUSTIFICATION OF THE CONSENSUS DOCUMENT

Lyme disease or Lyme borreliosis (LB) is a complex multisystemic process predominantly distributed in the northern hemisphere, transmitted by the bite of hard ticks of the *Ixodes ricinus* complex (*Ixodes ricinus*, *Ixodes persulcatus, Ixodes scapularis, Ixodes pacificus*) and caused by different genospecies of *Borrelia burgdorferi* sensu lato (here after *B. burgdorferi*)[1-3]. In Europe, *I. ricinus* is the main vector[2,3].

Much of the clinical spectrum of LB, such as acrodermatitis chronica atrophicans (ACA), erythema migrans (EM) and neurological manifestations including Garin and Boujadox meningopoliradiculitis, and the so-called Banwarth´s syndrome had already been described in Europe since the late XIX and early XX centuries. In addition, its bacterial etiology and tick transmission had also been suspected. This infection, however, aroused great medical and social interest as a result of the description in the US in the 70s[4,5] and of the discovery of its etiological agent in the 80s[6], with gradual increase in the description of patients until becoming the most frequent tick-borne disease (TBD) in the northern hemisphere[7].

The diagnosis of LB may be easy in patients bitten by ticks, who develop the typical clinical manifestations of the infection, such as EM, in an endemic area for LB. But, sometimes, and despite the fact that there are other clinical manifestations suggestive of LB (e.g. meningorradiculitis and lymphocytic meningitis with facial nerve paralysis), these manifestations may be caused by other agents and processes, and a microbiological confirmation is required[2,8,9]. To complicate the diagnosis, many patients do not remember the tick bite, which can often go unnoticed because it is painless and in areas not accessible to sight. In addition, clinical reports of LB include nonspecific clinical manifestations such as prolonged asthenia, myalgia, arthralgia, and lack of concentration, among others, that, taken away from the appropriate clinical-epidemiological environment, can lead to confusion and misdiagnosis.

For many years, and this is still the case for most Public Network Centres in Spain, the microbiological criteria recommended by Health Agencies and Scientific Societies competent in the subject (e.g. Centers for Disease Control and Prevention (CDC), European Society of Clinical Microbiology and Infectious Diseases (ESCMID), Infectious Diseases Society of America (IDSA) and others) have been applied[10-19]. But in recent years, 'other techniques' that have not been validated by these Agencies and Societies have appeared, leading to the diagnosis of LB in patients without clear epidemiological and clinical criteria. In the opinion of many experts, and this is reflected in most clinical guidelines and consensus documents, many of these techniques have only originated confusion, without meeting the requirements of sensitivity and specificity to establish a correct diagnosis of LB[19-21].

Another problem, which adds difficulty to the diagnosis, is to differentiate an active infection from a past infection, and the high prevalence of antibodies against *B. burgdorferi* in endemic areas[22]. To complicate the issue, the literature includes cases of *B. burgdorferi*-infected patients who do not develop a measurable humoral immunity response, and patients with decreased levels of antibodies that may not be detected after an initial period of detection[12,23]. The culture of *B. burgdorferi* has also been described in patients with persistent nonspecific clinical manifestations after having received adequate antimicrobial treatment for LB, although this is exceptional[24].

For the above-mentioned reasons and taking into account that every day it is more frequent that we are consulted by patients who have been diagnosed of LB without meeting the required clinical-epidemiological and microbiological criteria and sometimes they are subjected to prolonged treatments not based on scientific evidence, the Spanish Society of Infectious Diseases and Clinical Microbiology (SEIMC) has considered the need to update and prepare a Consensus Document with other Scientific Societies such as the Spanish Society of Neurology (SEN), Spanish Society of Pediatric Infectology (SEIP), Spanish Society of Rheumatology (SER), Spanish Society of Immunology (SEI) and Spanish Academy of Dermatology and Venereology (AEDV) involved in the diagnosis and management of the LB.

2. METHODOLOGY FOR THE EVALUATION OF THE DOCUMENT

After contacting with the different Spanish Scientific Societies and experts in the field, and with the aim already proposed of agreeing on some useful recommendations for the management of patients affected by LB, an exhaustive bibliographic search was proposed on the state of knowledge of the infection by *B. burgdorferi* s.l. and LB in PubMed. Given that the bibliographic search with the words "Lyme disease" or "Lyme borreliosis" or "*Borrelia burgdorferi*" retrieved more than 15,000 references, it was decided that each expert would narrow the field and choose the most relevant bibliographic citations taking into account previous consensus documents and other recommendations of Health Agencies and Scientific Societies and relevant abstract books of Conferences. As in Spain there are none wide and updated reviews of the topic, it was also considered interesting to carry out an exhaustive review about the epidemiology and history of LB in Spain.

Since available guidelines and consensus documents have been recently published and they can be easily consulted[13-19], in our case, we have chosen to use the degree of consensus between Societies and experts' signatories of this document for the final recommendations.

3. MICROBIOLOGICAL CHARACTERISTICS OF *Borrelia burgdorferi* s.l. AND GENOSPECIES OF *Borrelia burgdorferi* PRESENT IN SPAIN

Bacteria of the genus *Borrelia*, along with the genera *Spirochaeta, Cristispira* and *Treponema*, fall within the Phylum XV Spirochaetes phyl nov., Class I Spirochaetia class. nov., Order I Spirochaetales and Family I Spirochaetaceae, and comprise 43 pathogenic species for birds and mammals, including humans. They have a characteristic spiral shape with size between 0.2 and 0.5 μm in diameter and between 3 and 33 μm in length. They have an external cell envelope, a cytoplasmic membrane, periplasmic flagella (from 15 to 20) and a protoplasmic cylinder. The flagella fulfill the functions of skeleton and mobility and are subproximally anchored in the bacterial body, and are located in the periplasmic space. They are Gram-negative and microaerophilic bacteria. According to the analysis of the 16S rRNA gene and other conserved genes such as the *flagelline* gene, and considering the ecological characteristics, *Borrelia* spp. are divided into two large groups, *B. burgdorferi* s.l. comprising the bacteria that cause LB, and a second large group of bacteria that cause recurrent fever[25].

A striking feature of *B. burgdorferi* s.l. is the small size and structure of its genome. This is composed of a linear chromosome, unusual in bacteria, of approximately 1 Mb and several plasmids, linear and circular, which vary in number up to 21. The guanine-cytosine ratio ranges from 23% to 32%. Another characteristic is the large number of lipoproteins that it expresses, and that are mostly encoded by plasmids, such as the 6 proteins in the outer envelope (OspA to OspF) and a variable expression protein called VlsE, which play an important role in the patient's immune response. There is an absence of genes that encode proteins that lead to cellular biosynthesis reactions, which limits the metabolic capacity of *B. burgdorferi* and turns these bacteria into obligated parasites that depend on their hosts for their nutritional support. However, the borrelias of this group grow in a highly enriched liquid medium called Barbour-Stoenner-Kelly (BSKII) at 30-34 °C in microaerophilic environment, dividing every 8-12 h during the logarithmic phase of their growth[25].

The 5S-23S rRNA intergenic space has been used to classify closely related genospecies of the *B. burgdorferi* s.l. complex (Table 1). Although the complex currently comprises 21 different genospecies, only *B. burgdorferi* sensu stricto (s.s.), *Borrelia afzelii, Borrelia spielmanii, Borrelia garinii* and *Borrelia bavariensis* are considered to be of pathogenic relevance to humans. Despite cases of LB caused by *Borrelia valaisiana, Borrelia lusitaniae,* and *Borrelia bissettiae* have been described, their pathogenic ability has been questioned and their description is occasional. *Borrelia mayonii* has been recently incorporated in the Americas[26].

There seems to be a tropism of different genospecies by different organs associated with plasmid variations. Thus, *B. afzelii* predominantly appears in dermatological manifestations such as ACA, *B. garinii* and *B. bavariensis* seem to present greater tropism by the nervous system, *B. burgdorferi* s.s. by the articular system, while *B. spielmanii* has been isolated exclusively from EM. Anyway, LB is a dynamic process with different clinical manifestations in different organs and systems, depending not only on genospecies, but also on time[2,3].

The concept of genospecies has generated controversy among various authors throughout history, and this has been greatly exacerbated in recent years. Some argue that genospecies are nothing more than genetic variations of the same species, while others criticize that genetic classification is not relevant in many cases from an ecological point of view. In 2014, Adeulu and Gupta proposed the reclassification of spirochaetals of the genus *Borrelia*, so that those that cause LB (*B. burgdorferi* s.l. complex) would be renamed '*Borreliella'*, while those that cause recurrent fevers would continue with the name '*Borrelia'*, based on the results of their analysis of genetic markers 'unique' in the species[27]. Recently, detractors of this new classification have asked the Judicial Commission to support the rejection of the name '*Borreliella'* and all its combinations, based on the violation of several principles of the Code of International Nomenclature of Prokaryotes, such as, among others: endangering human health and patient safety by the confusion they create in the medical and scientific community and its possible consequences on medical coverage, avoid unnecessary creation of new names and that names should not be changed without sufficient justified reasons[28]. This controversy has led to the non-adoption of the new nomenclature

in most published works since its proposal, adding more confusion to the chaos that this has caused. It is therefore imperative that an appropriate taxonomic committee be involved in resolving this debate.

Table 1: Genospecies of *Borrelia burgdorferi* sensu lato complex

Genospecies	Year	Vector	Main host	Pathogenicity for humans	Epidemiological distribution
B. afzelii	1994	*I. ricinus* *I. persulcatus*	Micromammals	+++	Europe, Asia
B. americana	2010	*I. ricinus* *I. minor*	Rodents, birds	-	USA
Candidatus B. andersonii	1995	*I. dentatus*	Cotton-tailed rabbit	-	USA
B. bavariensis	2013	*I. ricinus* *I. persulcatus*	Micromammals, birds	+++	Europe, Asia
B. bissettiae	2016	*I. pacificus* *I. spininpalpis* *I ricinus*	*Neotoma fuscipes* (dusky-footed woodrat)	+	USA, Europe
B. burgdorferi sensu stricto	1984	*I. scapularis* *I. pacificus* *I ricinus* *I. persulcatus*	Mammals, birds	+++	USA, Europe
B. californiensis	2016	*I. jellisoni* *I. spinipalipis* *I. pacificus*	*Dipodomys californicus*	-	USA
B. carolinensis	2011	*I. minor*	*Peromyscus gossypinus, Neotoma floridana*	-	USA
B. chilensis	2014	*I. stilesi*	*Oligoryzomys longicaudatus*	-	Chile
Candidatus B. finlandensis	2011				
B. garinii	1992	*I. ricinus* *I. persulcatus*	Birds	+++	Europe, Asia
B. japonica	1994/3	*I. ovatus*	Rodents	-	Japan
B. kurtenbachii	2014	*I. scapularis*	Rodents	-	USA & Europe
B. lusitaniae	1997	*I. ricinus*	Lizards	+	Europe

B. mayonii	2016	*I. scapularis* *I. pacificus*	Mammals	++	USA
B. sinica	2001	*I. ovatus*	*Niviventer confucianus*	-	China
B. spielmanii	2006	*I. ricinus* *I. persulcatus*	Garden dormouse	+++	Europe
B. tanukii	1997/6	*I. tanuki*	Vole	-	Japan
B. turdi	1997/6	*I. turdus*	Unknown	-	Japan, Europe
B. valaisiana	1997	*I. ricinus* *I. granulatus* *I. columnae*	Birds	?	Europe, Japan, Taiwan, Korea
B. yangtzensis	2015	*I. granulatus*	Rodents	-	Asia

4. LYME BORRELIOSIS IN SPAIN. EPIDEMIOLOGY, DISTRIBUTION AND FEATURES OF *Ixodes ricinus*

The first confirmed LB patients in Spain date back to the 1980s when the first cases of neuroborreliosis and EM were described[29-31]. Subsequently, other isolated cases were reported[32-36], and small/medium series of patients, either from collaborative studies or from single centers, were published[37-40], thus broadening the spectrum of clinical manifestations and making clear that the LB is common in Spain, affecting children and adults of both genders. Most cases have been reported in the northern half of the Iberian Peninsula.

In addition to the description of clinical cases, studies of seroprevalence of antibodies were carried out in different population groups showing that the infection is frequent in people who develop outdoor activities (hikers, hunters, fishermen, environmental workers and others) in which antibodies can be found in a high percentage. The risk of infection also increases with age. In fact, seroprevalence have ranged between 0-40% depending on the area, population and used technique[22,41-53] (Table 2).

However, since LB is not a notifiable disease in our country, we do not have records or reliable data on the incidence of this disease. Most patients are diagnosed by physicians in the event of an EM or suspicion of clinical manifestations with nervous system involvement.

In Europe, according to data from 2006 (latest WHO update), 85,000 cases are reported annually; these being clearly lower than the real data, as it is not a notifiable disease[54]. There are other estimations between 60,000 and more than 200,000 cases per year, only in Germany[55]. In Spain, there are no real incidence data.

Table 2: Seroprevalence of *Borrelia burgdorferi* infection in humans (Spain)

Seroprevalence	Population type	N	Geographical area	Method	Cut-off value	Ref.
38%	Foresters and Rangers	42	La Rioja	IFA	≥ 1/128	41
5.8% 31%	Healthy population People bitten by ticks	500 38	La Rioja	IFA	≥ 1/128	22
29% 10% 1.3%	HIV infected people	72		IFA EIA WB	≥ 1/128	42
13.1%	Healthy population	298	Soria	IFA	≥ 1/256	43
16.4% 0%	Suspected LB Healthy population	354 150	Granada	WB		44
4.1%	Healthy population	98	León	IFA	≥ 1/128	45
25%	Forestry workers, 117; veterinarians, 52; shepherds, 18; apiculturists, 27; mushroom and truffle gatherers, 74; other outdoor activities, 14	302	Vizcaya	EIA WB	Negative control +3 SD	46
3.5%	Healthy population	1825	Madrid	IFA		47
4.4%	Patients (adults and children) admitted to the Hospital for surgical intervention not related to an infection.	203	Barcelona	EIA		48
3.7%	Suspected LB	936	Cartagena	EIA		49
4.4%	Healthy population	1429	Navarra	EIA		50
9.6% 4.3%	Suspected LB	623	Palencia, Burgos	IFA WB	≥256	51
13.2% 5.1% 16.2%	Healthy population Blood donors Suspected LB	1432	Asturias	EIA		52
7%	Forest rangers	100	Guadalajara	IFA IgG	≥256	53

Ref.: Reference; EIA: Enzimo-immuno-assay; IFA: indirect immunofluorescence assay; LB: Lyme borreliosis; N: number of subjects; WB: western blot.

As previously stated, the vector of the *B. burgdorferi* s.l. infection and LB in Europe is *I. ricinus*. The first prevalence studies of *B. burgdorferi* in ticks were carried out at the beginning of the 90s[56,57] by means of immunofluorescence techniques and later, by PCR. Table 3 details the different studies carried out, showing very different prevalence depending on the area of Spain (0% to 48%), with higher prevalence when nymphs and adults are studied from the same area. So far, the genospecies *B. burgdorferi* s.s., *B. afzelii*, *B. garinii, B. lusitaniae, B. valaisiana* and *B. turdi* have been detected[56-71].

Table 3. Prevalence of *Borrelia burdorferi* infection in *Ixodes ricinus* from Spain.

Year	Area	Source	Technique	N	Tick stage	% of infection	Genospecies	Ref.
1990	La Rioja	Cow	IFA		Adults	11%	ND	56
¿¿	La Rioja Castilla y León	Cow Birds	IFA	2856	Adults Nymphs	14% 57%	ND	57
1992-1997	Basque Country	Vegetation	PCR	5452	Adults Nymphs	5% 0.8%	*B. garinii* *B. burdorgferi* s.s. *B. valaisiana* *B. lusitaniae*	58
1997-2002	Castilla y León	Humans	PCR	1329	Adults Nymphs	6.1% 0.9%	*B. lusitaniae* *B. garinii* *B. valaisiana*	59
1998-2000	Basque Country	Vegetation Rodents	PCR		Adults		*B. burgdorferi* s.s. *B. garinii* *B. valaisiana* *B. afzelii*	60
2002-2003	La Rioja	Vegetation	PCR	25	Nymphs	48%	*B. afzeli* *B. garinii* *B. valaisiana*	61
2003-2005	Basque Country	Vegetation	PCR	288	Adults	1.7%	*B. afzelii* *B. garinii*	62
2004	Asturias	Vegetation	PCR	448	Nymphs	4%	ND	63
2009	La Rioja	Birds	PCR	181	Nymphs Larvs	10.5% 7.8%	*B. garinii* *B. valaisiana*	64

2009-2011	La Rioja	Birds	PCR	17	Nymphs	40%	*B. turdi*	65
2009-2016	La Rioja, Basque Country, Navarra, Cantabria	Vegetation	PCR	652	Nymphs	4.1%	*B. afzelii* *B. garinii* *B. lusitaniae* *B. valaisiana* *B. burgdorferi* s.s.	66
2012-2014	Asturias	Vegetation	PCR	845	Adults	6.1% 1.4%	*B. afzelii* *B. garinii* *B. lusitaniae* *B. valaisiana*	67
2014-2015	Iberian Peninsula	Dogs	PCR	147	Adults	2.7%	*B. afzelii* *B. garinii* *B. valaisiana*	68
2015	Galicia	Vegetation	PCR	1048	Adults Nymphs	24% 12.2%	*B. afzelii* *B. burgdorferi* s.s. *B. garinii* *B. lusitaniae* *B. valaisiana*	69
??	Galicia	Roe deer	PCR	3449	Adults Nymphs	0.4% 0.1%	*B. garinii* *B. valaisina* *B. lusitaniae* *B. afzelii*	70
2015-2017	Galicia	Vegetation	PCR	1056	Adults Nymphs	14.9% 10%	*B. afzelii* *B. garinii* *B. lusitaniae* *B. valaisiana* *B. burgdorferi* s.s.	71

Ref.: Reference; IFA: indirect immunofluorescence assay; N: number of subjects; ND: not determined; PCR: polymerase chain reaction

In 1992, García-Moncó *et al.* succeeded in cultivating *B. burgdorferi* s.s and in 2000, Escudero *et al*. cultured *B. garinii, B. afzelii, B. valaisiana*, and *B. lusitaneae* from *I. ricinus*[72,73]. In 1998, Oteo *et al*. isolated the first pathogenic *B. burgdorferi* strain in Spain, corresponding to *B. garinii* (RIOJA-1 strain), from a patient with an EM from La Rioja[74], and given the description of LB cases with clinical manifestations not-defining of LB only based on serological findings and without clear epidemiological antecedents, the "Lyme Disease Study Group of the SEIMC" established a definition of 'endemic area' to support the diagnosis[75].

In Spain, the number of diagnosed cases decreases from North to South with areas considered endemic, such as La Rioja, Navarra, North of Castilla y León, Asturias, Cantabria, the Basque Country and more recently, Galicia, where in the last decade a progressive increase in the number of reported cases has been observed[20,76-78].

As already noted, in the Iberian Peninsula, as in the rest of Europe, LB is transmitted to humans by the bite of hard ticks of the genus *Ixodes*, and specifically by *I. ricinus* (**Figures 1-3**).

Figure 1: Female *Ixodes ricinus* waiting on the grass.

Figure 2: Patient bitten by an adult female (A) and a nymph (B) of *Ixodes ricinus.* Note the concomitant presence of an erythema surrounding the tick caused by the local irritation of the tick saliva.

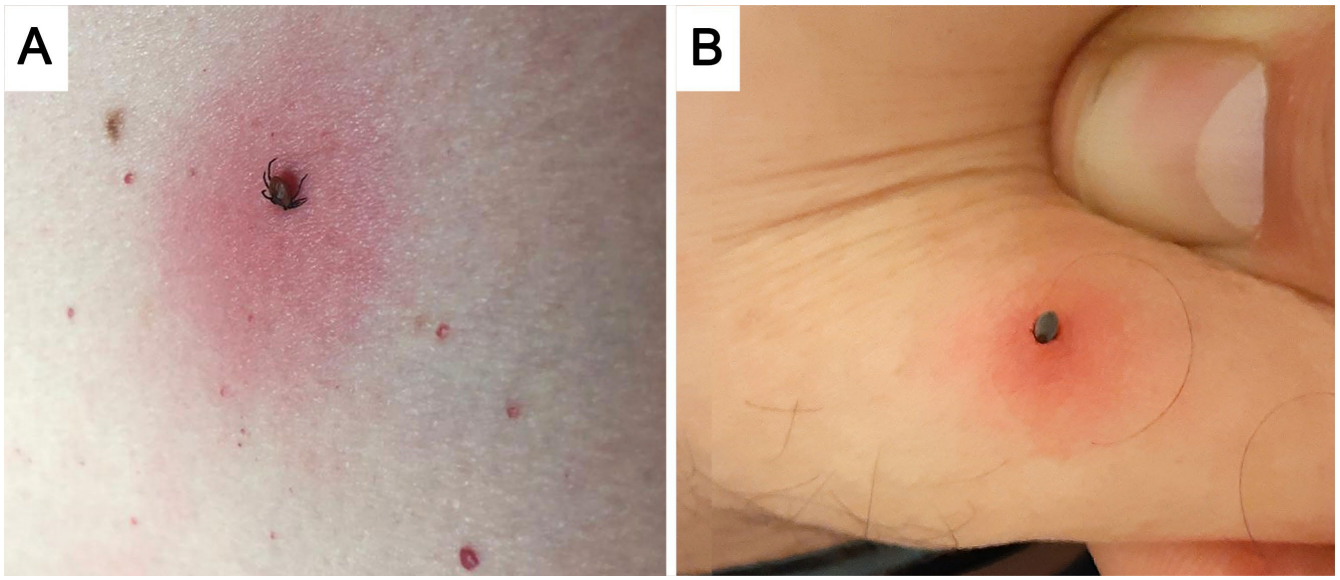
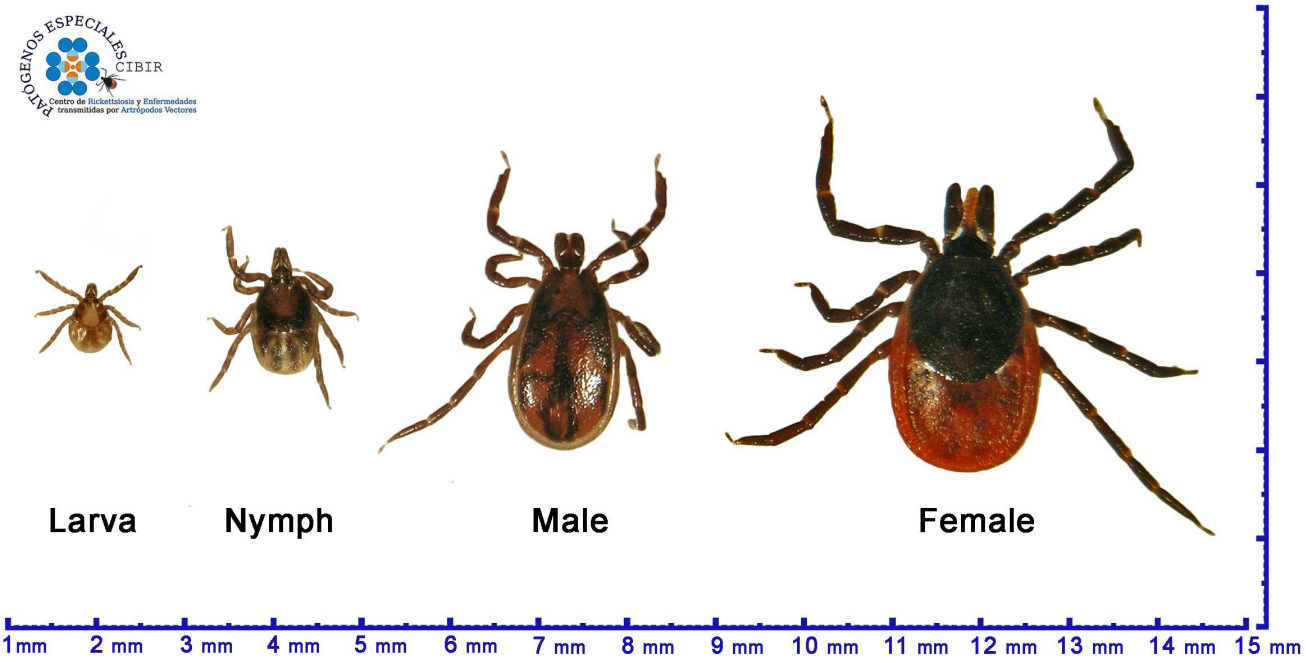


Figure 3: Different sizes and stages of *Ixodes ricinus.*



This species, particularly in its nymph stage, is the one that most frequently bites people in northern Spain[79]. *I. ricinus* is the tick species with the highest bacterial alpha-diversity (species richness) within the anthropophilic ticks from our environment[80]. In Spain, *I. ricinus* has been described not only as vector of *B. burgdorferi* s.l. (mainly, *B. garinii* and *B. afzelii*) but also as vector of other human pathogenic microorganisms, such as *Rickettsia monacensis*[81], *Rickettsia helvetica*[64,82], *Anaplasma phagocytophilum*[83,84], *Neoehrlichia mikurensis*[85], *Babesia* spp.[86,87], *Borrelia miyamotoi*[66] and of other bacteria species not implicated in human pathology, such as the recently cultured *Rickettsia vini*[88]. Despite *I. ricinus* is the vector of the TBE virus, it has not been notified in Spain up to date[89,90], and only imported cases have been detected.

I. ricinus is generally distributed in temperate deciduous forests and mixed forests with shrubs, thick undergrowth and a high degree of relative humidity (>80%). In figure 1, a female *I. ricinus* can be seen waiting for a host. In figure 3 the different stages of the tick and their sizes are detailed. They prefer areas with litter cover on the ground that provide protection against drought in summer and cold in winter, creating a humid microclimate. In rainy areas, it is easy to find these ticks in coniferous forests connected with grasslands where there are extensively exploited livestock and abundant cervids that acts as dispersers and amplifiers as well as wild fauna (micromammals), which act as reservoirs[91]. They can also live in urban and peri-urban environments. In areas such as La Rioja or Navarra, *I. ricinus* habitually lives in areas with a minimum altitude of 400 m and a maximum of 1,200-1,300 m. However, in western areas with the influence of the Atlantic Ocean and higher humidity, *I. ricinus* can be found from sea level to 2,000 m of altitude. In southern Spain, there are also areas where there are stable populations of *I. ricinus*, such as the `Parque de los Alcornocales´ in Cadiz, and the 'Doñana National Park'[20,92]. In the last decade, the distribution of *I. ricinus* (and the pathogens it transmits) continues to expand northwards in latitude and towards higher altitude areas throughout Europe[93-95]. The shorter and less severe winters in recent years

appear to have contributed to a greater abundance of *I. ricinus*, parallel to an expansion of its reservoirs and hosts. These factors and the phenomena of contact between ticks (co-feeding) seem to be responsible for the local variations in the prevalence of the different *Borrelia* spp. (and other microorganisms) in ticks[96]. *I. ricinus* are mainly active from spring to autumn, although we can find them active throughout the year depending on the factors mentioned above. Activity in spring is usually higher than in autumn (associated with higher temperature and photoperiod) with the exception of larvae, which show the opposite situation in some areas[92,97,98].

Other species of ticks such as *Ixodes hexagonus, Ixodes canisuga* and *Ixodes frontalis*, contribute to the circulation of *B. burgdorferi* in Spain[57, 65], although no cases of LB associated with their bites, which are rare in humans, have been reported.

5. CLINICAL MANIFESTATIONS OF LYME BORRELIOSIS

In most patients in whom *B. burgdorferi* causes disease, the clinical manifestations follow a chronological course that can be related to the pathogenesis and pathophysiological changes caused by the causative bacteria. As it is a dynamic process over time, it has been classified into different phases or stages[2] as detailed in table 4.

Table 4: Classification and main clinical manifestations of Lyme borreliosis

Phase	Clinical manifestations
Early localized	EM, lymphocytoma with or without lymphadenopathy
Early disseminated	EM multiple, disseminated lymphocytoma and/or early neurologic, cardiac and musculoskeletal manifestations. Ophthalmic manifestations.
Late	ACA, lymphocytoma, late neuroborreliosis, persistent or relapsing arthritis of more than 6 months

EM: Erythema migrans ACA; Acrodermatitis chronica atrophicans.

In this document, we have reviewed the state of the knowledge of LB clinical manifestations according to the organ or system affected.

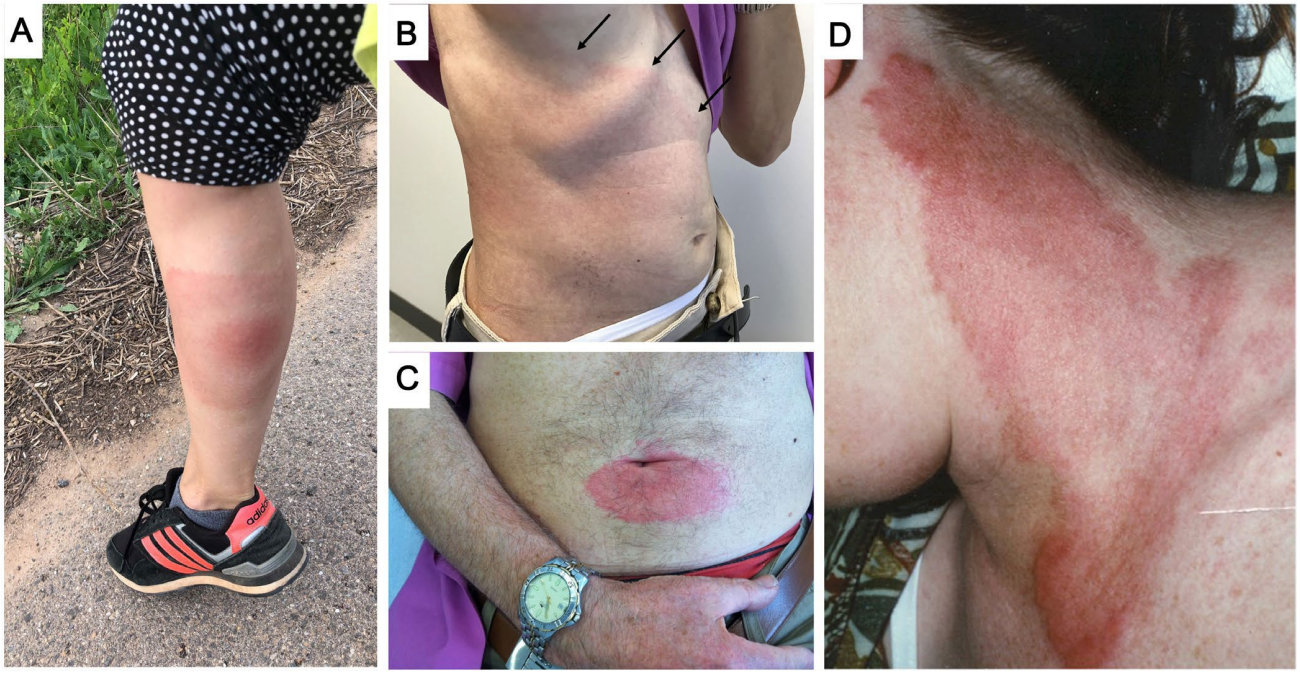
5.1. Skin manifestations

The skin manifestations are the most frequent and best documented, and can appear during all phases of the infection. The first descriptions date from the end of the 19th century in Europe (*Acrodermatitis chronica atrophicans* - Buchwald 1983)[99] and the beginning of the 20th century (*Erythema chronicum migrans* - Afzelius 1909)[100]. Later, other manifestations such as *lymphadenosis benigna cutis,* now called lymphocytoma associated with *B. burgdorferi,* were added[101].

5.1.1. Erythema migrans (EM)

The earliest and most typical clinical marker of LB in both, North America and Europe, is EM[102,103]. It is characterized by the development of a small erythematous macule at the point of the tick bite, which grows at the border and typically clarifies in the center, acquiring a targeted or annular appearance (**Figure 4A**), although it can also sometimes take other more atypical forms (**Figures 4B and 4C**).

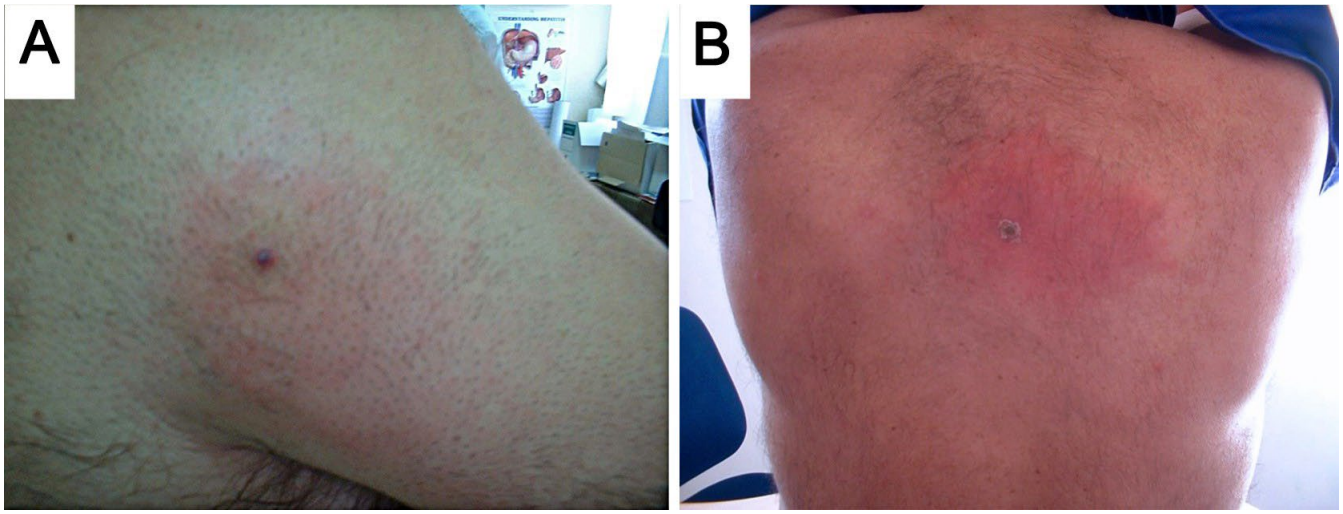
Figure 4: A) Typical erythema migrans (EM) with annular appearance on leg. B) Large EM of more than 6 weeks of evolution. C) EM without the typical annular appearance. D) EM in the early disseminated phase with satellite lesions.



If unrecognized and untreated, it can grow and become large, losing intensity in the tonality of the borders (**Figure 4B**). It appears a few days to 2-3 weeks after the bite and without treatment, it can take many weeks or months to disappear. This is the early localized phase of the infection. In some patients, the bacteria spread through the skin through the lymphatic vessels and similar lesions or satellite plaques appear, usually smaller in size, which are sometimes purpuric in appearance (**Figure 4D**). This is what we call multiple EM and corresponds to the early skin disseminated phase of the *B. burgdorferi* infection. EM is usually painless, although some patients report a certain stinging-itch in the area. The border of the lesion is usually sharp, unlike other cellulites. The clinical presentation of EM in children is similar than in adults, although EM is more commonly localized on the head or neck that in adults[104]. A variable percentage of patients with EM have arthromyalgia, low-grade fever and conjunctivitis[55,103,104]. The presence of fever and severe impairment of the general state, with or without other clinical manifestations or changes in blood parameters, should alert us to a possible co-infection by other agents transmitted by *I. ricinus* (in our media, *B. miyamotoi*, *A. phagocytophilum*, *N. mikurensis*, *Babesia* spp., *R. monacensis*). The differential diagnosis of EM in our environment should be made with other annular erythemas and DEBONEL (*Dermacentor-borne-Erythema-Necrosis-Lymphadenopaty*), caused by *R. slovaca* or *Ca.* R.

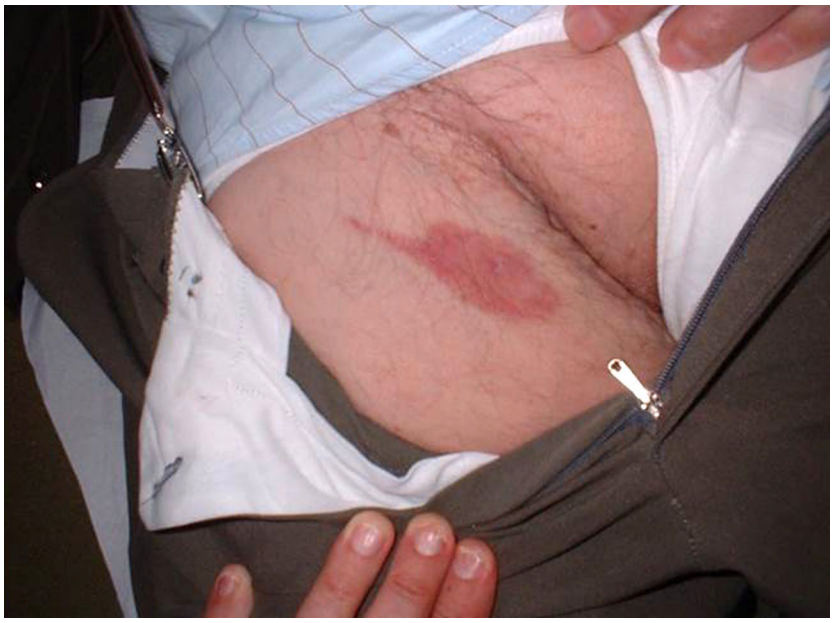
rioja, since when this infection is located outside the head, an EM-like appears but with central necrosis[105] (**Figure 5**).

Figure 5: Erythema migrans-like lesions with eschar in patients bitten by *Dermacentor marginatus* and caused by *Candidatus* Rickettsia rioja (A) *and Rickettsia slovaca* (B) (DEBONEL).



In travelers returning from certain US areas, it can be very difficult to distinguish between EM and southern tick-associated rash illness (STARI)[106]. EM must be also differentiated from the skin reaction caused by the tick-bite (saliva) (**Figure 2**) and sometimes the substances used to remove the tick (liquid nitrogen) can cause a reaction that may simulate an EM (**Figure 6**). That is why we do not recommend this practice.

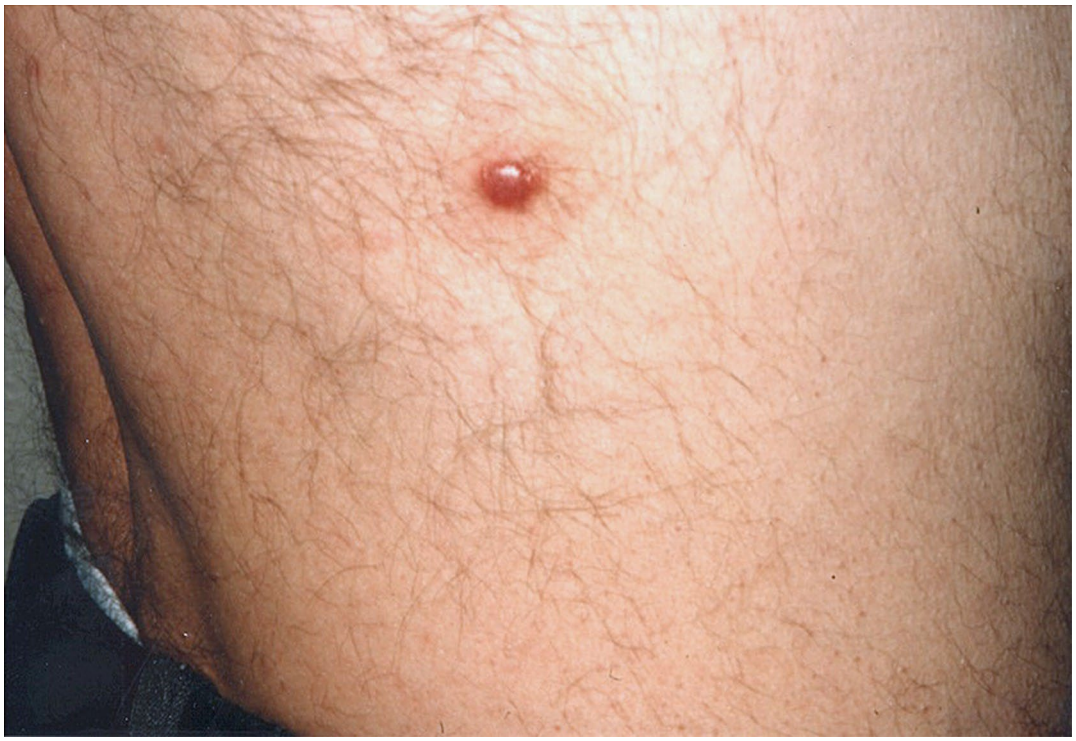
Figure 6: Erythema migrans-like lesion after removing a tick. A day before, a spray of liquid nitrogen was used to freeze the tick.



5.1.2. Lymphocytoma

In Central Europe, and associated with *B. afzelii* infection, and mainly in children, the so-called *Borrelia* lymphocytoma has also been described during the early localized phase, and less frequently in the disseminated phase. This rare manifestation is usually located on the earlobe, face, nipple or scrotum. It manifests as a well-located plaque or as a painless bluish-red nodule at the point of the tick-bite or at a distance that appears within weeks of contracting the infection. Microscopically, the architecture is made up of a dense lymphocytic infiltrate that must be differentiated from cutaneous lymphomas[55]. Although communicated, it is a rare skin manifestation in Spain[107] (**Figure 7**).

Figure 7: Borrelial lymphocytoma.



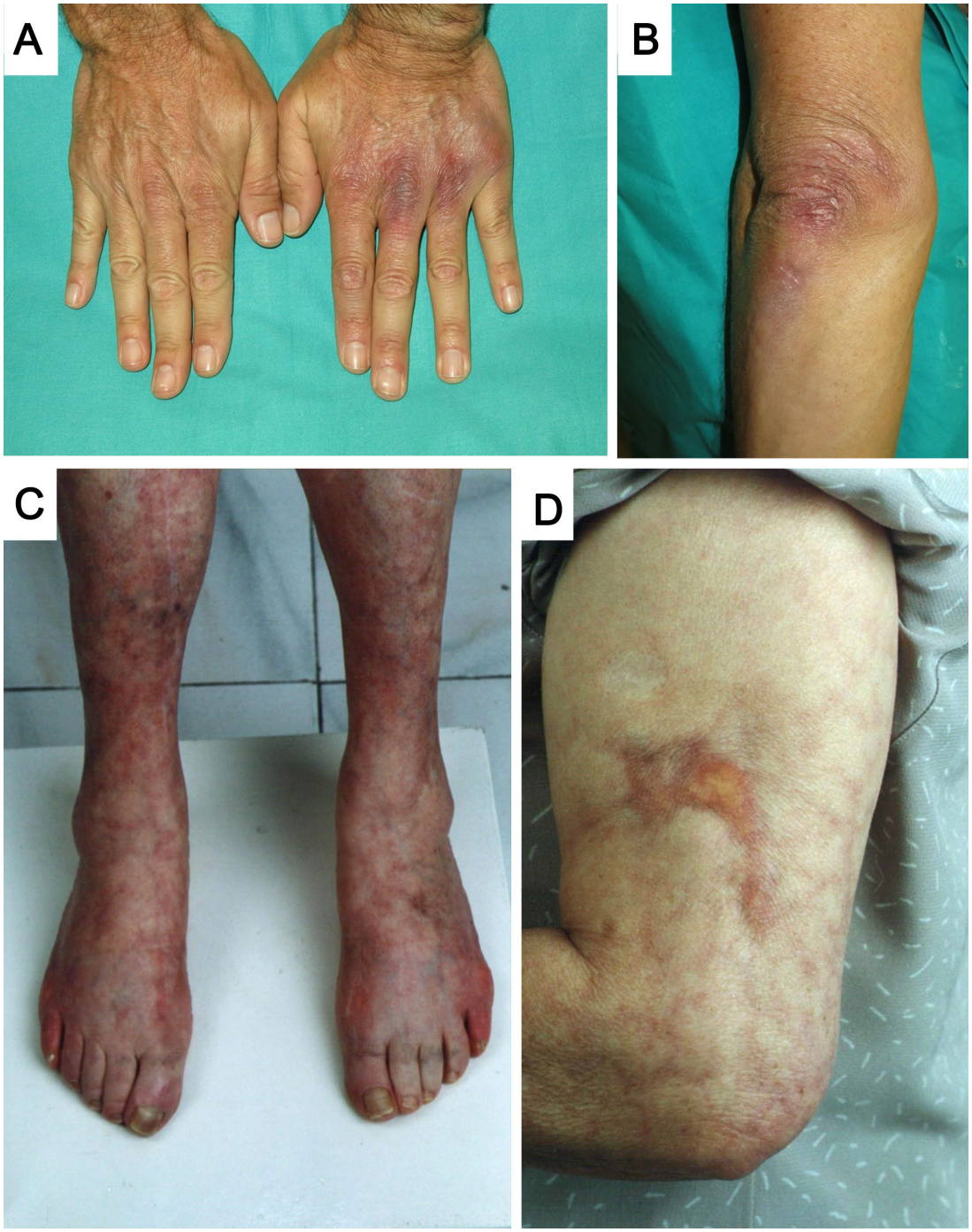
5.1.3. Acrodermatitis chronica atrophicans (ACA)

ACA is another cutaneous manifestation in patients with persistent infection (late stage), overall in Central Europe and much less frequent in Spain (**Figure 8**). In a large series recently published in Slovenia, authors conclude that ACA is typically caused by *B. afzelii* (also other genospecies can be involved) and usually affects old women, although it can be diagnosed in children. Clinical presentation depends on the duration of illness and probably on the *Borrelia* genospecies causing the disease[108]. It starts as a violaceous patch, usually located on the extensor surface of a limb. Periarticular nodules and cords can also be present (**Figure 8**). It progresses slowly without treatment, causing a skin atrophy, which will allow see the vessels of the skin. ACA is accompanied by a polyneuropathy in up to 50% of cases[55,109].

These three manifestations are clearly related to an infection with *B. burgdorferi*. The relationship between infection with *B. burgdorferi* and other dermatoses, especially morphea, lichen sclerosus, and interstitial granulomatous dermatitis is still debated.

Figure 8: Acrodermatitis chronica atrophicans (ACA) affecting left hand (A) and left elbow (B) with an underlying fibrous cord on the arm. ACA affecting low extremities (C) and an arm with a fibrotic nodule

(D).



5.2. <u>Neurologic manifestations</u>

The involvement of the central and peripheral nervous system (CNS and PNS) in *B. burgdorferi* infection, occurs in approximately 15% of infected patients, particularly in the early disseminated phase of the infection (weeks after the tick bite) and less often in later stages (2-3% of infected patients)[110-112]. The neurological manifestations in Europe and the US appear to be different in some aspects[113-115].

Lyme Neuroborreliosis is divided between early and late manifestations (duration of signs and symptoms for more than 6 months), as well as between CNS and PNS manifestations.

5.2.1. Early neuroborreliosis: The most common manifestations of early neuroborreliosis are cranial neuropathy (particularly facial palsy), lymphocytic meningitis, and radiculoneuritis, which can occur in

isolation or in combination, and these are known as the Garin-Bujadoux-Bannwarth syndrome, although unfortunately recent literature only mentions Bannwarth's syndrome. This condition occurs weeks after the appearance of the typical skin lesion (EM) or the tick bite, and is characterized by a severe, migrating radicular pain that can be accompanied by peripheral nerve paresis, often combined with uni- or bilateral (one-third of cases) facial palsy and cerebrospinal fluid (CSF) pleocytosis. Pain disappears after antibiotic treatment; however, the disorder spontaneously resolves in 5 to 6 months without therapy[116]. Patients presenting with facial palsy are commonly misdiagnosed as having Bell's palsy, and radiculitis may be mistaken for a herniated disc. The presentation in the periods of activity of *I. ricinus* as well as a history of tick exposure and a skin lesion compatible with an EM should alert to the clinicians.

Two recent retrospective studies in Denmark and Germany showed that the most common neurological disorder was radiculitis, present in 66% and 50% of patients, respectively, facial palsy in 43% and 25%, and meningitis in 10% and 6%[113,114]. Aside from radiculitis, patients with early neuroborreliosis may also have other forms of PNS involvement, including plexopathies and a more disseminated polyneuropathy or mononeuritis multiplex. In the US and Europe, facial palsy and lymphocytic meningitis are the most common early manifestations. Headache is the main complaint in meningitis, and fever and meningismus may be mild or absent. In untreated patients, recurrent attacks of meningitis may alternate with periods of remission. CSF analysis shows a moderate (around 100 cells/mm^3) lymphocytic mononuclear cell pleocytosis[117] sometimes with atypical features, resembling lymphoma with moderate protein increase, and normal glucose contents[35].

Occasionally, patients may have a stroke, likely secondary to infective endarteritis, in a similar way as it occurs in syphilis or tuberculosis. A few cases of retrobulbar optic neuritis, papillitis, neuroretinitis and ischemic optic neuropathy have been reported. Papilledema secondary to raised intracranial pressure in Lyme meningitis occurs in children, with few adult cases reported[111,112].

5.2.2. Late neuroborreliosis: This condition is much less common and may present as a peripheral polyneuropathy accompanying ACA, an almost exclusively European entity[109]. The neuropathy is predominantly sensory and tends to follow the topographical distribution of the skin disease.

Late CNS involvement may appear months to years after the disease onset, and it was initially known as chronic encephalomyelitis[118]. In addition to be very uncommon (less than 2% of all Lyme neuroborreliosis), it represents a controversial entity[119-122]. It is defined as continuous disease lasting more than 6 months and its diagnosis can be only made in the presence of suggestive neurologic symptoms, CSF pleocytosis, and intrathecal *B. burgdorferi* antibody production[123]. Patients complain of cerebral malfunctioning, particularly cognitive problems, or present spinal cord signs and symptoms, including paraparesis, ataxia, and bladder dysfunction. When there is CNS involvement, the CSF shows a lymphocytic pleocytosis (usually in hundreds of cells/mm^3), increased proteins and normal glucose. More details are discussed in the diagnosis section.

5.3 Musculoskeletal manifestations

Musculoskeletal manifestations in the context of LB are frequent. In fact, its importance has been reflected in the history of LB itself, which in the first descriptions in the US was called 'Lyme arthritis' (LA)[5]. The

prevalence of the joint and musculoskeletal manifestations is more frequent depending on the geographical area since the clinical spectrum is different between the American continent and Europe.

5.3.1. Arthritis

In Europe, oligoarticular arthritis and joint inflammation during the early disseminated phase seem to be less frequent than in the American continent, and in late stages, this arthritis is rarely resistant to antibiotic therapy and hardly related to autoimmune mechanisms. In the US, more than 10% of LA cases are resistant to treatment and associated to autoimmune mechanisms[3,20,102]. In Spain, reports of the classic form of LA are rare, probably related to the fact that *B. garinii* is the most frequent genospecies causing LB in Spain. Anyway, as it occurs with the cutaneous and neurological manifestations of LB, in Spain it is more frequent to diagnose this type of manifestations in north-western regions[20,124]. According to data from a recent study including patients who underwent serology tests and showed positive results in urban areas, the prevalence of LA was very low (6 cases out of 78 positive serologies from 574 samples)[125]. In the natural history of the disease, up to 45-62% of patients with untreated EM develop LA characterized as a monoarthritis or oligoarthritis. LA can be intermittent or persistent, frequently affecting the knee joint, although it can present as an asymmetric oligoarthritis[1-5,125].

In a study carried out from 2010 to 2016 in France, *Borrelia* was detected in 37 out of 357 (10.4%) synovial fluids tested by PCR. Patients' median age was 36 years (range 6-78) with 61% of men and 28% patients under 18. The presentation was monoarticular in 92% and the knee was involved in 97%. Contrary to the *Borrelia* genospecies distribution in European ticks, *B. burgdorferi* s.s. was the most prevalent species found in synovial fluid (54%) followed by *B. azfelii* (29%) and *B. garinii* (17%)[127]. In this series, despite proper antibiotic therapy, roughly one third of patients presented persistent inflammatory synovitis and a small proportion developed systemic arthritis[127]. Apart from the knee, it can also affect the shoulder, ankle, elbow, temporo-mandibular joint or wrist. The association with bursitis or inflammatory tendinopathy is usual. Less frequently, it affects more than five joints, mainly large. Inflammatory episodes, that begin acutely, can last from weeks to months, being much more frequent in untreated patients. It is common to present arthritis with clinical joint synovial fluid effusion, although the inflammation is not extremely painful except for loaded and pressured joints or in over-weighted patients[125]. If the inflammation chronically persists in correctly treated patients (10%), the concept of post-infectious LA appears. It is characterized by proliferative synovitis that persists ≥2 months after oral antibiotics or ≥1 month after, at least, two weeks of intravenous antibiotics, which may lead to joint dysfunction due to cartilage erosions and joint radiological progression. It is thought to be related to persistent immune activation rather than persistent infection status and is still discuss[128]. The study of synovial fluids reveals an inflammatory process with an elevation of the cell count between 10,000-25,000 cells/microL, increase of proteins, none specific, neither different from other infections.

The differential diagnosis includes all acute and chronic inflammatory processes, mainly monoarticular or oligoarticular, due to infectious agents and/or inflammatory and autoimmune diseases. Previous history of EM helps to focus the clinical picture of such arthritis and seek for LB. The serological response to *B. burgdorferi* is the main diagnostic test, but occasionally seroconversion does not occur until a few weeks later in cases of early disseminated infection. Patients with arthritis occurring in advanced stages of the

disease are usually seropositive for antibodies to *B. burgdorferi*.

The outcome of patients with early LB covers from a stage of full recovery to the development of autoimmune arthritis such rheumatoid arthritis or psoriatic arthritis, within months of treatment, likely occurring in patients with unique risk factors (psoriasis), considering the infection a potential trigger to chronic stages[129,130]. In the aforementioned French series, despite proper therapy, 34% of patients developed persistent synovitis for at least two months (median duration: 3 months, range 2-16). Among those, three patients developed systemic inflammatory oligo- or polyarthritis in previously unaffected joints with no signs of persistent infection (repeated PCR testing negative), which mandated disease-modifying anti-rheumatic drugs introduction, leading to remission[127].

Moreover, the presence of musculoskeletal manifestations of migratory type or recurrent arthralgia is very frequent in early stages (50-75%), and they are also present in patients in late stages of the infection[14]. They are unspecific, overlapping through the infectious disease course. Therefore, in the absence of other typical manifestations of the disease such as EM or meningoradiculitis, the presence of isolated arthralgias should not justify the investigation of LB (Consenus level: 9/9)

5.4 Cardiological manifestations

Cardiological manifestations in the context of LB can be observed in the early disseminated and late stages of infection, although their communication is much less frequent than skin, neurological or joint manifestations. This is because, in most cases, *B. burgdorferi* only causes self-limited atrioventricular conduction blocks (AV-B) that do not cause clinical manifestations or do so temporarily. According to the CDC, involvement occurs in only 1.1% of reported LB cases and it is more common in men in USA[131], although a study carried out in New York city with children suffering LB without symptoms of carditis, showed electrocardiographic alterations in up to 29%, most frequently AV-B grade I[132]. In Germany, it can be found in up to 10% of patients[133]. There are no data of prevalence in Spain, although members of this panel have observed asymptomatic AV-B in patients with early localized and disseminated forms of LB. Anyway, according to current opinion, there is acute, self-limiting Lyme carditis, and persistent Lyme carditis. Acute Lyme carditis mostly manifests as transient conduction disorders of the heart (e.g. AV-B I to III) or supraventricular and ventricular rhythm disturbances, pericarditis, myocarditis, and pancarditis in single cases that can be cause of cardiac failure and sudden death[134]. Usually these patients spontaneously recover within 3 to 7 days and thus, permanent pacemakers are not needed. Other authors reported that myocarditis is relatively frequent[135,136].

Persistent Lyme carditis is defined as a case of chronic heart failure confirmed by positive serology and endomyocardial biopsy. Tick-bites or EM are not always reported. Seropositivity and control of its specificity by western-blot (WB) are indicative but no an etiological proof. Even histological detection of spirochetes in endomyocardial tissue or cultivation of borrelia from endomyocardial biopsy are not final etiological proofs of the respective cardiac disorder. Those findings, however, are an indication for antibiotic treatment[137].

According to the recently published American guidelines[19], ECG should only be performed in patients with signs or symptoms consistent with cardiac involvement in the context of LB, including dyspnea, edema, palpitations, lightheadedness, chest pain and syncope (Consensus level: 9/9)

5.5. Other clinical manifestations

The development of other clinical manifestations accompanying the typical clinical manifestations of LB is relatively frequent.

5.5.1. Ophthalmic manifestations

Ophthalmic manifestations may occur in every stage of the disease. Conjunctivitis and episcleritis are the most frequent manifestations in early localized stage. Neuro-ophthalmic disorders and uveitis occur in the early disseminated stage, whereas keratitis, chronic intraocular inflammation and orbital myositis have been reported in the persistent stage of borreliosis[138]. In some cases, these ophthalmological manifestations may also be due to a Jarisch-Herxheimer-type reaction[139]. These ophthalmological manifestations are not specific of LB, and they do not require investigation of *B. burgdorferi* infection without a clear clinical-epidemiological history or out of the context of LB, since a positive result could be equivocal.

5.5.2 Psychiatric manifestations

Although psychiatric disorders may coexist in the course of LB, there is no recommendation to request microbiological tests to determine the state of infection by *B. burgdorferi* in patients with such disorders if they do not present other clinical manifestations suggestive of LB. The same occurs in children with attention deficits[19]. We refer the readers to the excellent and exhaustive reviews about LB previously cited herein, which mention other manifestations that may accompany the wide spectrum of clinical manifestations of *B. burgdorferi* infection[3,102].

5.5.3. Congenital Lyme disease

Vertical transmission of *B. burgdorferi* is a proven fact, although there are controversies regarding the risk of transmission and effects on delivery and fetus[140]. In 2018, Waddel *et al*. performed a systematic review of gestational LB and 59 cases were identified from 1969 to 2017[141]. Twelve cases were associated to miscarriage or fetal death; eight cases, with newborn death; and 16, with other post-delivery abnormalities, including syndactyly, respiratory distress and hyperbilirubinemia. One case described complete features of clinical and laboratory results consistent with vertical transmission of LB. They also summarized eight epidemiological studies comparing features or serology from pregnant women in endemic areas with non-Lyme pregnancies. The authors concluded that there was no association between gestational LB or surrogate measures of exposure and adverse birth outcomes. A meta-analysis of nine studies showed significantly fewer adverse birth outcomes in women treated for gestational LB compared to those who untreated during pregnancy, providing indirect evidence of association between gestational LB and adverse birth outcomes. Other risk factors investigated, such as trimester of exposure, acute *vs.* disseminated LB at diagnosis, and symptomatic LB *vs*. seropositive women with no LB symptoms during pregnancy were not significantly associated with adverse birth outcomes[141].

5.6 Post-treatment Lyme syndrome (PTLS)

Patients diagnosed of LB and correctly treated usually have a full recovery. Anyway, persistent neurologic deficits, such as facial paralysis or persistent pain can be observed in a low percentage of treated patients. Time to recovery can be longer in patients with late stages also. These facts should not be confounded with PTLS.

PTLS has been defined as persistent symptoms without objective manifestations that persist for at least six months after conventional treatment for LB has been completed[142]. These patients usually refer nonspecific symptoms, such as fatigue, arthralgia, myalgia, or perceived cognitive impairment. These symptoms should not be attributed to persistent active infection. In this context, serological tests should not be used as proofs of efficacy of the treatment since despite the fact that antibody titres usually decrease after treatment, patients can remain seropositive for years and this fact does not mean active infection[12,143].

If these symptoms persist after adequate treatment, several controlled studies have shown that immunocompetent patients do not benefit from retreatment or prolonged treatment[19,144-146]. In these patients, other possible causes of disease that justify the persistence of clinical manifestations should be sought (Consensus level: 9/9).

'Chronic Lyme Disease' is a term that creates great confusion and it is often used by some doctors and patients[147]. Most Health Agencies and Scientific Societies are against the use of this term, which is commonly used to define patients with nonspecific and persistent symptoms in whom no active infection is demonstrated, and even, in many cases, they never have had *B. burgdorferi* infection confirmed with tests recommended in this and other guidelines for the diagnosis of *B. burgdorferi* infection. These patients must be differentiated from patients with clinical manifestations of the late phase of LB with evidence of *B. burgdorferi* infection, and from patients with PTLS (Consensus level: 9/9).

6. DIAGNOSIS OF *Borrelia burgdorferi* s.l. INFECTION AND LYME BORRELIOSIS

6.1. Direct diagnoses

The accurate microbiological diagnosis of *B. burgdorferi* infection and LB is based on the demonstration of the presence of the agent in different biological samples by culture and/or visualization of *B. burgdorferi* in the affected tissues. These techniques require great technical and time-consuming dedication as well as trained staff and continuous quality controls, so they are usually only available in specialized laboratories. In addition, the culture is mainly sensitive in the early phase of the disease, in which the diagnosis is based on the epidemiological history and clinical manifestations. As the infection progresses over time and other organs and systems are affected, the sensitivity decreases. There are different culture media -usually liquid-, with incubations between 30-35ºC up to 12 weeks and in microaerophilia, such as the Barbour-Stoenner-Kelly (BSK) and its modifications, such as BSK-II or BSK-H, or the modified Kelly-Pettenkofer medium (KPM)[148-150]. However, this technique only has a high performance in skin samples (biopsies of ACA and EM)[151], decreasing its sensitivity when performed in sterile fluids such as CSF or synovial fluid in the early disseminated phase (eg: acute meningorradiculitis and arthritis), and even more in the late stages of the disease (e.g. persistent neurological syndromes). Regarding the visualization of *B. burgdorferi* s.l., the lack of specific antibodies for the development of immunohistochemistry techniques

also limits their use, although there are specific stains to demonstrate the presence of spirochetes in tissues (Warthin-Starry, modified Dieterle or modified Steiner silver stains)[152-154]. For all these reasons, the direct diagnosis is mainly based on molecular biology techniques (PCR assays in their different versions: conventional, real-time, isothermal, etc.). Their sensitivity at least overlaps with that of culture techniques[155]. They are faster, more affordable, and also allow us know the involved genospecies. Nevertheless, molecular tests are not standardized and partial fragments of a variety of chromosomal genes, such as *fla*, *p66*, 16S rRNA or plasmid-borne genes, such as *ospA*, *ospB*, *VlsE* or the 5S/23S rRNA intergenic spacer region can be used as PCR targets. It is worth-noting that plasmid-borne genes may yield false positive results since borrelias are able to shed blebs containing plasmids that dissociate from bacteria and may persist in tissues and body-fluids without active disease[156]. Therefore, detection based on chromosomal genes is recommended. Since their sensitivity can be lower, the use of two target genes is recommended[10]. Molecular detection of *B. burgdorferi* should be also performed with appropriate samples (e.g. blood and urine are not suitable materials for diagnosis)[157] and in specialized laboratories. PCR assays are useful in patients with skin manifestations, especially with EM, where the sensitivity is around 70%, according to a meta-analysis conducted by Ružić-Sabljić and Cerar (2017), with a better profitability in skin biopsies from ACA patients, where the diagnostic sensitivity reaches 75%[158]. However, molecular assays are not worthy in cases of EM since this skin manifestation is very specific and highly suggestive of LB. Synovial fluid is considered a valuable sample for the diagnosis of LA by PCR, with median sensitivity of 77.5%. It decreases up to 22.5% for CSF in neuroborreliosis, and it is 18% or even lower for blood, serum or plasma samples[158]. A negative PCR result does not exclude the possibility of LB. The sample should be quickly processed in the laboratory under optimized conditions (4-8ºC in less than 24 hours after the extraction) to obtain the highest yield for *Borrelia* detection[10]. Sensitivity of samples fixed in paraffin or kept for longer periods of time is reduced when compared with that of fresh or fresh/frozen specimens[159]. The specificity of positive results must be confirmed by identification up to genospecies level to reduce contamination risks. We recommend the use of molecular diagnostic tests in cases of suspicion of neuroborreliosis with CSF, ACA with skin biopsies and LA with synovial fluids, and always performed by specialized laboratories. (Consensus level: 9/9)

6.2. Indirect diagnoses

Due to the difficulties indicated, the most common and accessible diagnostic techniques are the serological ones to demonstrate the presence of antibodies against the causative agent. In this regard, it is worth recalling the kinetics of antibody response against *B. burgdorferi*. Thus, from the bite of the infecting tick to the development of the humoral immune response, a ‘window period’ or ‘serological silence’ passes, in which the presence of antibodies is not detected in the infected individual. In patients who develop EM as the first clinical manifestation of the disease, seroconversion occurs between two and four weeks after observation[143,160], being those who present localized EM, without systemic involvement, the ones with the lowest seroconversion rate. A negative serological result at an early stage does not necessarily exclude the diagnosis of BL. For this reason, to demonstrate *B. burgdorferi* s.l infection, the serological test should be repeated at least four weeks later. Anyway, it should be clear that in patients with an EM it is not necessary to confirm the presence of antibodies against *B. burgdorferi* to make the diagnosis of LB. Early

antimicrobial therapy may abrogate the antibody response, resulting in seronegativity, although evidence is contradictory[143,161].

In general, the number of *B. burgdorferi* proteins that are recognized by the immune system of the infected individual significantly increases during the course of the disease. In the early stages of infection, the first proteins to be recognized are OspC (Outer surface protein C), flagellin and BbK32 (Fibronectin-binding protein)[162] that the bacterium expresses early to evade immune mechanisms. In relation to IgM and IgG antibodies produced during infection, IgM occurs in the EM phase only in half of patients in the first two to four weeks of disease development, so 50% of them are negative to this antibody. If the patient progresses towards the appearance of a second phase of systemic involvement (e.g.: with associated myalgias and arthralgias), IgM production reaches a peak at six-eight weeks and then gradually lowers the titre after three months[163]. However, there may be patients who remain IgM positive for a long time (up to ten years after the infection has passed and been correctly treated)[164]. In addition, IgM can be positive in cases of syphilis, infection by Epstein-Barr virus, HIV, systemic lupus erythematous and other connective diseases and immunological processes, due to cross-reaction[165]. Cross-reactions with poorly characterized circulating antigens have also been described in some pregnant women and even in healthy individuals[166,167]. This is due to some antigens of the bacterium may share epitopes similar to these other infectious agents and to the individual's self-antigens. The development of IgM is followed by an increase in IgG production. This response of polyclonal IgG is directed to numerous proteins of the microorganism such as the aforementioned BbK32, OspC, flagellin and VlsE (Vmp-like sequence E). Subsequently, other IgG antibodies are directed against other proteins such as p58, DbpA (Decorin-binding protein A) and BmpA (*Borrelia* membrane protein A), among others[168]. In the late and evolved phases of the disease, normally, although not absolutely, IgM turns negative and an increase in IgG is observed compared to a greater number of antigens.

For all the above, we do not recommend giving a diagnosis of LB based on an isolated positive IgM value, except in early phases, with typical manifestations of the disease and always in an adequate epidemiological environment. Thus, support for the microbiological diagnosis of LB should preferably be performed by IgG measurement (consensus level 9/9).

The most commonly used serological methods are the enzyme immunoassay (EIA or EIA based), indirect immunofluorescence assay (IFA) and immunoblot or Western Blot (WB).

6.2.1 EIA: Different techniques use this approach. The most common is ELISA (enzyme-linked immunosorbent assay), and it can be automated, allowing the processing of a large number of samples and better standardization. Other equivalent techniques, such as ELFA (Enzyme Linked Fluorescent Assay), CLIA (ChemiLuminiscence ImmunoAssay) and MMIA (Multiplexed Microbead ImmunoAssay) have been also developed. The antigens used in all these commercially available techniques can be of four types: 1) Sonicates of the whole bacterium, obtained by culture technique; 2) Purified native antigens; 3) Recombinant antigens (OspC, OspA, BmpA, VlsE); 4) Synthetic peptides such as C6 (extracted from a region of the VlsE) or pepC10 (extracted from the OspC).

The use of sonicates of whole bacterial cells entails the presence of a high number of antigens, many of them of low specificity, which implies a high risk of generating cross-reactions[169-171]. Some ELISA tests

may use a mixture of recombinant antigens with whole cell lysates, which may increase sensitivity in the early phase of LB while maintaining specificity[172,173]. With the emergence of new *Borrelia* spp. as human pathogens in Europe (e.g. *B. miyamotoi*) the serodiagnosis is even more difficult since ELISA and WB tests designed for the diagnosis of LB may also show cross reactions against *B. miyamotoi* antibodies due to *B. burgdorferi* and *B. miyamotoi* share proteins such as FlaB, GroEL and BmpA (P39)[174,175]. If these assays are also cross reactive against other *Borrelia* spp. such as *B. mayonii* is unknown. Up to our knowledge, this species belonging to the *B. burgdorferi* s.l. complex has not been found in Europe.

In addition, the sensitivity of commercial EIA based techniques varies depending on the phase of the patient's disease. Thus, in the phase of localized EM without systemic involvement, the sensitivity is around 54%, in neuroborreliosis it reaches 81%, 96% in arthritis and 97% in ACA. Specificity is generally considered between 90% and 97% in healthy controls[176].

6.2.2. IFA: Another option for the first step of serodiagnosis is the IFA technique. The antigens used are complete bacteria fixed on a slide, alone or in combination with immunodominant antigens, such as VsIE or OspC. Although at first this was the most used or reference technique, today it is used less often than the EIA techniques, since it is not automatable and it is subject to the observer's subjectivity (with the consequent lower reproducibility inter-laboratories)[177]. In addition, the interpretation of results is difficult because the optimal dilution of the cut-off point is not standardized[44]. For these reasons, as stated in the German laboratory guidelines[178], we do not consider IFA to be the most appropriate serological technique for screening (Consensus level: 9/9).

6.2.3 WB: It is used for confirmation of EIA or IFA tests. For its interpretation, qualitative criteria have been proposed (assessing as positive the infection by *B. burgdorferi* when certain bands appear)[179] of a quantitative type (assessing not so much the type of bands but the greater or lesser number of them)[180] or a combination of both[181]. The limitations, as with other serological techniques, are that the simple positivity of bands in the WB can indicate a past contact with the microorganism, an active acute infection, a persistent infection, a cross-reactivity with other microorganisms (particularly in the presence of isolated bands against the flagellin protein), or be the result of a monoclonal or polyclonal stimulation nonspecific B lymphocytes in the course of infections by lymphotropic viruses, such as the VEB. For these reasons, we repeat that the serological diagnosis should be always made under a clinical suspicion of LB and within the appropriate epidemiological context (Consensus level: 9/9).

The incorporation of the C6 peptide or the VlsE protein to the EIA techniques has been proposed in America as a unique and sufficient technique for the microbiological diagnosis or as a second confirmation test ignoring the use of a WB[19]. In Europe, two-tier laboratory assay strategy based on a highly sensitive screening EIA based as first step, followed by a highly specific immunoblot test (WB), as confirmation for positive or equivocal cases, continues to offer better profitability[13,15,18,178]. These differences are due to the fact that in Europe the presence of various genospecies involved in LB (*B. garinii, B. afzelli, B. burgdorferi* s.s.) can generate antigenic polymorphisms, complicating the serodiagnosis based on ELISA C6, unlike in America, where *B. burgdorferi* s.s. is the only species involved in LB[182].

Taking into account the comments and experience, in Europe the recommended serologic diagnosis of

LB consists of an EIA based technique followed by WB[13,15,18,178]. It must be mentioned that these tests must be performed only in cases of clinically suspected LB (for patients with signs indicated under the clinical case definitions) in an adequate epidemiological environment, with the exception of EM, to avoid over-testing with the subsequent unnecessary costs[13]. This panel assumes the recommendation with a level of consensus: 9/9.

As already mentioned above, the serology results will depend on the stage of the disease. Thus, in early LB, whose only manifestation of the disease may be the presence of EM, or in the short-term acute neuroborreliosis, serology can be negative in up to 60% of patients. In these cases, with high suspicion of LB and negative serology results, it is advisable to repeat it in three or four weeks to check if there is seroconversion[18]. (Level of consensus: 9/9).

A properly treated LB does not preclude the subject from being reinfected after a new tick bite. In these cases, when antibodies from the first infection may continue to exist, serodiagnosis is complicated for the clinician. When we have a patient with a possible reinfection with previously positive serology, it is advisable to do a serology in the new acute phase of reinfection, repeating it three-four weeks later in order to detect any increase in the antibody titre or modifications in the pattern of WB bands, with respect to the first infection (Consensus level: 9/9).

In case of suspected neuroborreliosis, blood serology is insufficient since, even if positive, it would establish the diagnosis only in a few cases (e.g.: the development of facial paralysis in a child after a recent bite of *I. ricinus* in an endemic area with a positive serology result against *B. burgdorferi* is highly suggestive of neuroborreliosis). CSF analysis should be performed, since on rare occasions patients may show IgG antibodies in the CSF in the absence of a peripheral response. In neuroborreliosis there is a lymphocytic pleocytosis, sometimes with the presence of plasma cells, a highly suggestive finding. The spirochetal invasion of the CNS results in the local production of CXCL13, a B-cell attracting chemokine with the subsequent intrathecal production of specific antibodies. The demonstration of intrathecal antibody production is highly indicative of neuroborreliosis and relies on measuring anti-IgG *Borrelia* antibodies in both CSF and serum, and referring it to the total albumin or immunoglobulins G in both samples[111]. The formula usually employed is:

Antibody Index = Concentration (U/ml) of specific IgG antibodies in CSF/Concentration (U/ml) of specific IgG antibodies in blood serum / Total concentration (mg/L) of IGG en CSF/ Total concentration (mg/L) of IgG in blood serum.

An antibody index greater than 1.3 indicates positivity for intrathecal synthesis[183,184]. In addition, the CSF can be processed for culture and molecular techniques.

6.3 Other techniques for the diagnosis of infection with *B. burgdorferi* s.l. and LB

There are other techniques that have not been approved by any scientific agency or society as valid for the diagnosis of LB and that, for this reason, are discouraged:

6.3.1. Determination of CD57+

CD57+, also called HNK-1, LEU-7 or L2, is a sulfated carbohydrate molecule, with a molecular weight between 100 and 115 kD. It has been defined as a Natural Killer (NK) lymphocyte marker, although it is

only expressed in a percentage of lymphocytes and is also expressed in T lymphocytes, especially in the senescence phase or 'exhausted lymphocytes'[185]. Based on a study by Stricker and Winger, a low CD57+ cell count (mean 30±16 cells/ml) was associated with the controversial term chronic LB[186]. The authors studied 73 patients with LB who started late antibiotic treatment and found an increase in the count of these cells after therapy (66±39 cells/mL). However, this study suffers from several biases, such as the low number of patients included, biases in the presentation of results, non-monitoring of expression kinetics in patients after treatment, non-validated control groups, and absence of a clear case and treatment definition. Other researchers, such as Marques *et al*. found no significant differences in CD57+ between nine patients with post-Lyme syndrome versus 12 patients cured of LB and the control group consisting of nine healthy volunteers[187].

The elevation or decrease of the CD57+ marker has been associated with HIV, B hepatitis, C hepatitis, measles, parvovirus 19 and cytomegalovirus (CMV) infections[188], and non-infectious pathologies, such as multiple sclerosis, systemic lupus erythematosus, rheumatoid arthritis, dermatomyositis, polymyositis, ankylosing spondylitis or chronic fatigue syndrome, among others. Alterations in the expression of this marker have been also detected in different types of cancer[189].

Based on these studies and publications, the CD57 marker does not seem a useful parameter even after antibiotic treatment or persistence of symptoms. To date, none studies have proven the usefulness of this test or its sensitivity and specificity (Consensus level: 9/9)

6.3.2. ELISPOT Interferon-γ Test (IFN-γ)

It is based on the release of IFN-γ from samples of peripheral blood leukocytes that are stimulated with antigen/s of the microorganism, in order to explore the stimulation and activation of T lymphocytes against the different *B. burgdorferi* genospecies.

Several studies have shown the elevation of IFN-γ levels in patients with early, late/evolved LB and post-Lyme syndrome. The sensitivity of this test during the early phase of the disease ranges from 36% to 69%[190]. In another study in which this test was performed for patients who had a positive ELISA C6 test, sensitivity was increased to 83%[191]. Also, in this unique study, the decrease of this parameter was verified for patients undergoing antibiotic therapy for LB, and no other study has demonstrated the agreement between the serological tests and the concentration of IFN-γ. The specificity of the test is highly variable among studies according to the chosen population. Similarly, cross-reaction with other spirochetes and lack of reproducibility has been demonstrated[192]. The determination of this molecule in other samples (synovial fluid, CSF, skin biopsies, etc.) has not shown that it can be a useful diagnostic tool.

6.3.3. IFN-α

There are studies, such as that of Jacek *et al*. (2013)[193] that have shown an increase in IFN-α levels for patients with post-Lyme syndrome, which may suggest the existence of immune-mediated processes in patients with persistent symptoms, which may contribute to the immunopathology of the disease. These same authors demonstrated how treatment with beta-lactam antibiotics did not modulate the activated

immune response. As with the previous test, more studies are needed to demonstrate the clinical utility of this parameter.

Therefore, currently the ELISPOT IFN-γ test and the IFN-α should not be used in clinical practice until other research studies prove their usefulness (Consensus level: 9/9).

6.3.4. Lymphocytic proliferation test

It serves to assess the lymphoproliferative response of peripheral blood mononuclear cells against *B. burgdorferi* antigens. The results are expressed based on a Stimulation Index (SI). If the SI is greater than ten, the test is considered positive and if it is less than ten, negative. At present, no study has been able to demonstrate its clinical utility, obtaining low specificities and sensitivities, so that the diagnostic guidelines do not contemplate this test[13]. (Consensus level: 9/9)

6.3.5 CXCL-13 marker

This is an immunological marker (chemokine) that can be studied in CSF when suspected neuroborreliosis, also known by other names such as BCA-1 or B cell-attracting chemokine (B cell-attracting chemokine-1), and BLC or B lymphocyte chemoattractant. Its primary function is to attract B lymphocytes. It is expressed in high density in organs such as the spleen, lymph nodes, liver and digestive system. In relation to LB, this molecule is becoming one of the most promising diagnostic tools. Its greatest usefulness is within the diagnosis of early neuroborreliosis and several studies have demonstrated its elevation in the CSF of these patients[194-196]. It is detected before the antibodies in the CSF and also its concentration decreases in a short period of time once the antibiotic treatment is established, so it can also be used for monitoring it. It has a sensitivity of 89%-97% and a specificity of 96%. One of its drawbacks is that there is currently no established value as a cut-off point, with each laboratory having its own, so the interpretation can be variable inter-laboratories. Another drawback is that it can also rise in other inflammatory diseases of the CNS such as some viral meningitis, neurosyphilis, cryptococcosis or even lymphoma in CNS, which forces to discard these for a correct interpretation of this marker. This panel recommends this determination for the diagnosis and follow-up of LB with caution until there is no more data and its specificity is better known (Consensus level: 9/9)

6.3.6. There are other **markers** that are currently being evaluated, such as the determination of **CCL-19**[197], **apolipoprotein B-100**[198,199] **antibody-free chains (kappa and lambda) or the determination of total IgM and albumin** in the CSF of patients with probable neuroborreliosis. However, none has shown to be more promising than CXCL-13, as the range of positivity in patients presenting with other non-Lyme neuro-inflammatory diseases is higher, leading to low specificity[21]. Techniques such as either dark-field or focus floating microscopy are not recommended for diagnostic purposes[15,20] (Consensus level: 9/9).

7. TREATMENT

The antimicrobial treatment of LB has not changed in recent decades and it is based on the treatment of the infection by *B. burgdorferi* depending on the stage of the disease and the organ and/or system affected[200,201].

To our knowledge, there are no large clinical trials with an appropriate design and enough number of patients to support the recommendations with high level of scientific evidence in some cases, so many recommendations are fundamentally based on the few clinical trials and meta-analysis studies collected in the scientific consensus recommendations published by other scientific societies[15,17,19,55,202]. The choice of the drug will depend on the age, history of allergies, pregnancy, intolerances, or sun exposure given the possibility of photosensitivity with doxycycline. Tables 5 to 12 schematically show the drugs and recommended doses in each of the processes associated with LB.

We are aware of the controversy with the term "chronic Lyme" and its treatment[203,204]. Since the members of this panel do not consider other LB forms than those developed in the different sections of the text, and reject the term “Chronic Lyme Disease” related to a persistent infection by *B. burgdorferi* resistant to conventional treatment, we will not make recommendations on this aspect. Anyway, all the members of this document are against carrying out prolonged treatments with antibiotics and/or their combinations in patients who suffer non-specific clinical manifestations such as asthenia, arthralgia, lack of concentration, etc., with the exceptions that appear in the text for the simple fact of having suffered a previous infection by *B. burgdorferi* s.l. (Consensus level: 9/9)

Most LB patients respond to antimicrobial treatment in a timely manner, depending on the type of clinical manifestation and/or affected organ or system, although in patients under special conditions, such as those undergoing immunosuppressor treatments, anti-TNF, hematological malignancies or in elderly patients, the response may be slower and sometimes patients have to be retreated[205-208].

Another aspect that we want to highlight is that this panel recommends the use of doxycycline over other therapeutic options, when appropriate. Consideration should be given to the good penetration of this antibiotic into the CNS and other tissues due to the possibility of spirochete dissemination. Unlike the recently published American Guidelines, which consider the preferential treatment with intravenous beta-lactams in the treatment of neuroborreliosis[19], doxycycline can also be considered as an alternative treatment to intravenous beta-lactams for the treatment of CNS infections with meningeal involvement. In fact, in Europe, doxycycline is considered the treatment of choice if there are no parenchymal complications[15,17,209,210]. (Consensus level: 9/9)

In addition, doxycycline has the advantage of being active and of choice against other microorganisms transmitted by ticks that occasionally can co-infect the patient, such as *A. phagocytophilum, B. miyamotoi* and *Rickettsia* spp. that are circulating in our environment and are transmitted by *I. ricinus*. It is only recommended avoiding doxycycline in pregnancy and lactation, when the risk benefit must always be assessed[211,212]. Regarding the use of doxycycline in children, the American Academic of Pediatrics recently wrote: ‘Clinical use of tetracyclines in children younger than eight years has been limited due to the known binding to teeth and bones in young children that permanently may stain teeth. However,

doxycycline, a second-generation tetracycline, has not been shown to cause tooth staining in young children'. 'Doxycycline can be used for short durations (e.g. 21 days or less) without regarding patient age'[213].

Another factor to consider when prescribing an antimicrobial treatment against LB is that although treatments are generally well tolerated, depending on the phase of the disease, more than 15% of patients may experience a Jarisch-Herxheimer reaction consisting of a transient exacerbation of symptoms during the first 24 hours of treatment[200].

7.1. Early localized phase

7.1.1. Erythema migrans

There are some randomized clinical trials comparing the efficacy of different treatments in this situation. Results may vary between those performed in Europe or in the US, because of the design and to the fact that the *Borrelia* genospecies involved may be different. Anyway, and based on these clinical trials, the treatment of choice in this situation is an oral regimen with doxycycline, amoxicillin or cefuroxime axetil[214-217] as is showed in table 5. This panel, when no contraindication, preferably recommends the use of doxycycline for 10-14 days, both in children and adults (Consensus level: 9/9).

If a beta-lactam from those specified (equally effective) is chosen, it should be prolonged for a minimum of 14 days. The use of macrolides (azithromycin, clarithromycin or erythromycin) as first-line drugs is generally discouraged, leaving them as an alternative in cases where doxycycline, amoxicillin or cefuroxime cannot be used. In the case of the use of azithromycin, some authors recommended prolonging the treatment for seven days[218]. In pregnant patients, the use of ceftriaxone is the recommended option[219]. (Consensus level: 9/9).

Table 5: Treatment of erythema migrans in the early localized phase without other associated symptoms.

Drug	Adult dose	Child dose	Duration
Doxycycline	100 mg orally BID	4 mg/kg/day orally in two divided doses (maximum 100 mg per dose)	10 days (10-21 days)
Amoxicillin	500 mg orally TID	50 mg/kg/day orally in three divided doses	14 days (14-21 days)
Cefuroxime axetil	500 mg orally BID	30 mg/kg/day orally in two divided doses (maximum 500 mg per dose)	14 days (14-21 days)
Azithromycin	500 mg orally OD	5-10 mg/kg/day orally (maximum 500 mg per dose)	5 days (5-10 days)

BID: one doses every12h; TID: one doses every 8h; OD: one doses every 24h.

7.1.2. Borrelial Lymphocytoma

The pattern for borrelial lymphocytoma is similar to that used in EM[15], although a retrospective study of 144 adult patients treated with these guidelines has shown that 9.7% of the patients required retreatment because the lesion persisted after one month. Subsequently, it disappeared in all cases[220]. The recommended treatment is showed in table 6. It must be considered that there are no consensus among Societies since French guidelines recommend 14 days[15] and other guidelines such as the German ones recommend 21 days[55].

The duration in days of the treatment recommended in the table should be considered according to the severity and persistence of the clinical manifestations. Most patients treated with the recommended regimen present a complete resolution of the signs and symptoms in the following 20 days, avoiding the progression to other phases of the disease. As in other infectious diseases, some patients present subjective symptoms (headache, musculoskeletal pain, arthralgia or fatigue) that can persist for weeks or months after treatment. These symptoms usually resolve spontaneously in the following months and do not require sustained or repeated antibiotic treatment, as they are not due to the persistence of the infection. However, if the appearance of other clinical manifestations (e.g.: fever) is observed despite treatment, co-infections with other tick-borne agents (*A. phagocytophilum, Babesia divergens, B. miyamotoi*) should be ruled out. (Consensus level: 9/9)

It should also be considered that infection with *B. burgdorferi* and LB do not leave permanent immunity and LB may be suffered in more than one occasion (very rare). In that case, the patient will be treated under the same recommended guidelines[15]. (Consensus level: 9/9)

7.2. <u>Early disseminated phase</u>

7.2.1. Multiple Erythema Migrans

The recommended treatment is oral doxycycline for ten to 21 days, with the same considerations made in localized EM. Prolongation of treatment for more than ten days will be based on accompanying signs and symptoms[209,211]. The doses and duration of the treatment against multiple EM with associated flu-like symptoms and Borrelial lymphocytoma are showed in table 6.

Table 6: Treatment of multiple erythema migrans in early disseminated phase with associated flu-like symptoms and/or solitary or disseminated lymphocytoma.

Drug	Adult dose	Child dose	Duration
Doxycycline	100 mg orally BID	4 mg/kg/day orally in two divided doses (maximum 100 mg per dose)	14 days (10-21 days)
Amoxicillin	500 mg orally TID	50 mg/kg/day orally in three divided doses	14 days (14-21 days)
Cefuroxime axetil	500 mg orally BID	30 mg/kg/day orally in two divided doses (maximum 500 mg per dose)	14 days (14-21 days)
Azithromycin	500 mg orally OD	5-10 mg/kg/day orally (maximum 500 mg per dose)	7 days (5-10 days)

BID: one doses every12h; TID: one doses every 8h; OD: one doses every 24h.

7.2.2. Early neuroborreliosis

Clinical Practice Guidelines for therapeutic management of Lyme neuroborreliosis have been recently published by the Infectious Diseases Society of America, American Academy of Neurology and American Collage of Rheumatology[19], and the European Federation of the Neurology Societies (EFNS)[123]. German and French Guidelines have been also recently published[15,17].

Using an intravenous beta-lactam (ceftriaxone, penicillin or cefotaxime) for the treatment of these conditions has been the classic recommendation, and this option continues to be the one recommended by the American guidelines. In Europe, the new guidelines recommend the use of oral doxycycline as long as there are no parenchymal complications at the brain or spinal level or the clinical manifestations are very severe[210].

The recommended treatment for isolated facial paralysis without signs of meningeal involvement is oral doxycycline for a minimum of 14 days and a maximum of 28 days, although a clinical trial did not show differences in the response related to the duration of treatment[222]. In any case, as specified in previous paragraphs, the history of allergies, oral tolerance, pregnancy and lactation should be taken into account. The minimum duration of treatment should last at least 14 days in children and adults[15,17]. Adjunctive corticosteroids neither improve nor impair the outcome for patients with LB peripheral facial palsy treated with doxycycline[223]. As also specified in previous paragraphs, doxycycline is the elective antibiotic

treatment for the remaining neurological manifestations of the early phase, and as an alternative, a beta-lactam by intravenous route at the doses and with the duration specified in the table 7. In case of parenchymal involvement, the recommended treatment is an intravenous beta-lactam as is showed in table 7. (Consensus level: 9/9)

Table 7: Isolated facial palsy, or involvement of other cranial nerves with or without associated meningitis or polyradiculoneuropathy without parenchymal involvement and with parenchymal involvement*.

Drug	Adult dose	Child dose	Duration
Doxycycline	100 mg orally BID	4 mg/kg/day orally in 2 divided doses (maximum 100 mg per dose)	14 days (14-28 days)
Ceftriaxone*	2 g intravenous OD	80 mg/kg/día intravenous OD (maximum 2 g/day)	14 day (14-28 days)
Cefotaxime*	2 g intravenous TID	150-200 mg/kg/day intravenous divided in 3-4 doses (maximum 6 g/day)	14 days (14-28 days)
Penicillin G*	20 million Units intravenous divided in 6 doses	200,000-400,000 U/kg/day IV divided in 6 doses (maximum 20 million/day)	14 days (14-28 day)

BID: one doses every 12h; OD: one doses every 24h; TID: one doses every 8h.

7.2.3. Carditis

Asymptomatic AV-B with a PR interval of less than 300 milliseconds observed with relative frequency in the early stages of the disease does not require antimicrobial treatment different from that of the process itself. Patients with myopericarditis or those with severe or potentially severe involvement should receive intravenous antibiotic treatment at the doses and for the duration specified in Table 8. This can be simplified to the oral route (doxycycline, amoxicillin or cefuroxime axetil) once the blockage is resolved and/or clinical improvement occurs until completing a cycle of 21-28 days[19]. In patients with symptomatic bradycardia that cannot be managed with drugs, the American guidelines recommend the use of temporary pacemakers[19]. This panel agrees with these recommendations. (Consensus level: 9/9).

Table 8: Carditis in uncomplicated patient PR <300 ms and *carditis with first degree AV-B with PR >300 ms or 2/3 degree AV block or myocarditis.

Drug	Adult dose	Child dose	Duration
Doxycycline	100 mg orally BID	4 mg/kg/day orally in two divided doses (maximum 100 mg per dose)	14 days (14-21 days)
Amoxicillin	500 mg orally TID	50 mg/kg/day orally in three divided doses	14 days (14-21 days)
Cefuroxime axetil	500 mg orally BID	30 mg/kg/day orally in two divided doses (maximum 500 mg per dose)	14 days (14-21 days)
Ceftriaxone*	2 g intravenous orally OD	80 mg/kg/day intravenous orally (maximum 2 g/day)	14 days (14-28 days)

BID: one doses every12h; TID: one doses every 8h; OD: one doses every 24h.

Treatment of the other manifestations accompanying the early disseminated phase, such as the possibility of acute arthritis, should be carried out following the scheme in Table 7.

7.3 Late phase

7.3.1. Arthritis

In the case of arthritis, to prolong treatment with oral doxycycline, amoxicillin or cefuroxime for up to 28 days at the doses specified in table 9 is recommended. Some patients with sustained synovitis refractory to antibiotic treatment may benefit from the use of disease-modifying antirheumatic drugs, such as methotrexate or arthroscopic synovectomies[15,17,19].

(Consensus level: 9/9)

Table 9: Treatment of persistent arthritis.

Drug	Adult dose	Child dose*	Duration
Doxycycline	100 mg orally BID	4 mg/kg/day orally in two divided doses (maximum 100 mg per dose)	28 days
Ceftriaxone	2 g intravenous orally OD	80 mg/kg/day intravenous orally OD (maximum 2 g/day)	28 days
Amoxicillin	500 mg orally TID	50 mg/kg/day orally	28 days

BID: one doses every12h; OD: one doses every 24h; TID: one doses every 8h.

7.3.2. Acrodermatitis chronica atrophicans (ACA)

Treatment with oral agents is recommended as showed in Table 10. Doxycycline or amoxicillin for 30 days are the recommended ones[55]. When ACA is accompanied by involvement of the nervous system (usually as axonal polyneuropathy with predominant sensory symptoms), intravenous therapy with ceftriaxone or other beta-lactam should be used[55]. (Consensus level: 9/9)

Table 10: Treatment of acrodermatitis chronica atrophicans with or without associated polyneuropathy.

Drug	Adult dose	Child dose*	Duration
Doxycycline	100 mg orally BID	4 mg/kg/day orally in two divided doses (maximum 100 mg per dose)	28 days
Amoxicillin	500 mg orally TID	50 mg/kg/day orally in three divided doses	28 days
Ceftriaxone	2 g intravenous orally OD	80 mg/kg/day intravenous OD (maximum 2 g/day)	28 days

BID: one doses every12h; TID: one doses every 8h; OD: one doses every 24h.

7.3.3. Late neuroborreliosis

As detailed in the corresponding section for this phase, different neurological manifestations have been described, such as subacute encephalopathy, mononeuritis multiplex, peripheral axonal sensory neuropathy or encephalomyelitis, among others. These pictures are very rare and avoidable with effective treatment in previous stages of the disease. There are excellent manuscripts that have exhaustively reviewed their therapeutic approach. The EFNS recommends treatment of these conditions with intravenous ceftriaxone for three weeks[123]. The same therapeutic option is recommended by the American Academy of Neurology (AAN), although they do not specify the duration of treatment[224]. German guidelines recommend the same scheme for two-three weeks[17], while French guidelines recommend doxycycline and ceftriaxone as an alternative for three weeks[15]. This panel, as in the previous sections, chooses to recommend doxycycline as the first option and ceftriaxone as an alternative depending on the severity of the clinical picture and accompanying manifestations (e.g.: ACA and polyneuropathy), as showed in Table 11. As an adjunct to antimicrobial therapy, accompanying symptoms should be treated. Rehabilitation treatment and psychological support to patients are sometimes needed. (Consensus level: 9/9)

Table 11: Treatment of late neuroborreliosis.

Drug	Adult dose	Child dose	Duration
Doxycycline*	100 mg orally BID	4 mg/kg/day orally divided in two doses (maximum 100 mg per dose)	21 days (14-21days)
Ceftriaxone*	2 g intravenous OD	80 mg/kg/day intravenous OD (maximum 2 g/day)	21 days (14-21 days)

BID: one doses every12h; OD: one doses every 24h.

* In case of coexistence of ACA, 28 days.

7.4. Post-Lyme syndrome. Chronic Lyme borreliosis.

The prescription of an adequate treatment, under the recommendations established in the text, allows the control of the infection with cure for a very high percentage of patients. For patients treated in the early phase of the disease, cure usually occurs within three weeks whereas in the late-phase of the disease, the response is usually slower. Antibiotic treatment may fail, although this situation is rare and it is usually due to problems with adherence or absorption of antibiotics rather than to the existence of antibiotic resistance of *B. burgdorferi*. For patients with the so-called post-Lyme syndrome, there is some controversy. Studies showing no effect on such symptoms after prolonging the duration of the antibiotic treatment, repeating it or carrying out cycles with antibiotics, have been carried out[144,145,225-229]. This

approach is not recommended in any guideline. However, some authors advocate prolonging treatment in case of persistence of symptoms and evidence of coinfection by other tick-borne agents[203]. Anyway, the issue draws great controversy.

The members of this panel, until there is more scientific evidence, and since the persistence of *B. burgdorferi* infection after adequate treatment has not been demonstrated, are positioned not to use prolonged treatments or cycles or combinations of antibiotics in these cases. (Consensus level: 9/9).

We have often observed that prolonged treatment with doxycycline improves subjective symptoms in patients with post-Lyme disease or with other persistent inespecific symtoms of different diseases. This could be due to the inhibition effect of doxycycline metalloproteases rather than to their antimicrobial effect[230].

8. PROPHYLAXIS

Prophylaxis of LB is based on pre-exposition measures to avoid the bite of the vector and post-exposition measures.

8.1. Pre-exposition measures

The best method to avoid LB is preventing tick bites. In the table 12, the general recommendations for preventing LB are showed. This implicates preventing tick exposure by avoiding tick-infested areas during the periods of *I. ricinus* activity in Spain[231]. In case of going to the countryside, being keeping to the center of trails could minimize contact with adjacent vegetation where ticks are more abundant. The use of protective clothing that limits the contact of ticks with the body can be very effective to avoid tick attachment. It is advisable wearing light-colored clothing to detect the arthropod before attached to the skin, cap, long trousers tucked into the socks, long-sleeved shirt tucked into the trousers and do not wear sandals or open-toed shoes. It is desirable to inspect for unattached ticks on clothing because they can turn into a later tick-bite as well as washing clothes in hot water and dry clothing on high heat after outdoor activities. Bathing may also wash off unattached ticks[231]. The use of repellents has also demonstrated the decrease of tick-bites incidence when applied to clothes and/or bare skin. A good repellent should be effective against various arthropods, no irritating after topical administration, with pleasant odor or odorless, persistent after washing and economic. Recommended repellents for the prevention of tick-bites are DEET (N,N-diethyl-meta-toluamide), picardin, ethyl-3-(N-n-butyl-N-acetyl) aminopropionate (IR3535), oil of lemon eucalyptus (OLE), p-menthane-3,8-diol (PMD), 2-undecanone, or permethrin[19,231]. All of them can be applied to both skin and clothing, except permethrin which must be only applied to clothing due to its toxicity. When clothes are sprayed with permethrin (0.5%) or made with pretreated, permethrin-impregnated material provides high effective protection against tick-bites[232,233]. Its use on clothing could be effective for up several weeks and even supports the washings reducing significantly tick-bites and tick-borne pathogen transmission[232,234]. In general, efficacy and duration of repellents depends on the concentration used providing greater and/or longer periods of efficacy those products with higher concentrations[235]. However, DEET optimal concentration range varies from 15 to 33%. Products

containing >50% do not offer a significant increase in protection time over lower concentrations[236]. DEET, which is available in a wide variety of topical formulas providing up to 12 hours of protection, has been shown to be the most effective and with the broadest spectrum repellent[19].

Despite these products are reasonably safe to use, many people develop certain toxicity. This fact has misguided in the use of repellents based on natural products as garlic, citronella, eucalyptus oil, geranium oil, lavender oil or Alaska yellow cedar oil (e.g., citriodiol or p-menthane-3,8-diol available on the market as a tick repellent) but there is not enough evidence and their effectiveness has to be demonstrated. The Spanish Association of Pediatrics as well as the American Academy of Pediatrics (AAP)[237] and the CDC only recommend DEET for children at least two months of age, although it is desirable to be avoided as much as possible in children under two years of age[238]. The American Association of Pediatrics and the CDC do not recommend OLE and PMD for children <three years of age[19]. The contact between humans and parasitized domestic animals could increase the risk of a tick-borne disease acquisition. Thus, the most effective preventative measure arises in the use of effective long-acting acaricides in pets as permethrin, amitraz or fipronil or lindano[239,240].

The members of this panel assume these recommendations to avoid tick-bites (Consensus level: 9/9).

Other measures based on controlling and reducing ticks and tick-infected populations to reduce the number of human tick-bites and human diseases include physical, mechanical and biological strategies and are beyond the scope of these guidelines.

Vaccines for preventing LB were developed in past and only available in USA but to date commercial vaccines are not available for humans[241,242]. New technologies based in the new RNA vaccines developing antibodies against tick-saliva components have shown 'in vitro' activity to reduce *B. burgdorferi* infection.

Educational programs could be a good tool to decrease the risk of acquiring a tick borne infectious disease increasing people confidence and likelihood to practice precautionary behaviors[243].

This panel recommends education programs in schools and recreational or professional associations (hunters, mountaineers...) that instruct in the prevention of tick bites, how to recognize them and ways of extraction (Consensus level: 9/9).

Table 12: General recommendations to prevent Lyme borreliosis.

- Do not go off the trail when walking in areas where there are ticks.
- Use clothes that cover exposed areas of the body (cap, long trousers tucked into the socks, long sleeved shirt into the trousers and appropriate footwear).
- Wear light-colored clothing to detect ticks before they attach.
- Use tick repellents.
- Inspect the body after being in an outdoor area where ticks are abundant.
- Remove the tick with tweezers as soon as possible when detected.
- Take doxycycline in certain circumstances after tick-bite.
- Observe the site of the tick attachment for up to six weeks.

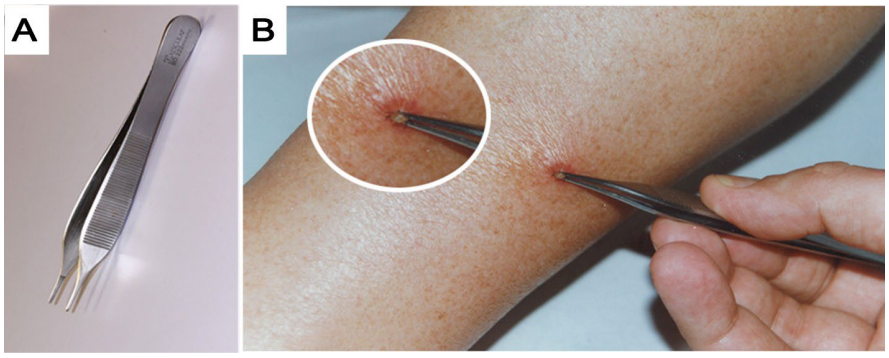
8.2. Post-exposition measures

If a patient is bitten by a tick, we must proceed to extract it and the use of antibiotic prophylaxis with doxycycline should be considered.

8.2.1. Tick removal

Despite wearing appropriate clothing, tick bites can occur and they are usually painless, making important to do an exhaustive exploration of the entire body in order to look for any attached ticks and remove them. Removal of the attached ticks must be done as soon as possible since it is accepted that 36-48 hours for *B. burgdorferi* transmission are needed, and the risk increases with longer attachments. Some European studies suggest the transmission of *B. burgdorferi* within 24 hours of attachment of *I. ricinus* ticks [244,245]. The use of tweezers or forceps to remove ticks significantly decreases the risk of complications associated to the tick-bite or the infection with the microorganisms they transmit[246]. The correct extraction of ticks should be done using thin-tipped tweezers or blunt, rounded forceps introducing them between tick head and the skin to grasp the mouth parts of ticks intact, if possible, and pulling the tick straight upward with steady pressure, perpendicular to the skin[247]. Other tick removal devices have been shown being useful for removing ticks[248-251]. If after the extraction any part of the tick is retained in the skin it should be advisable to perform a biopsy of the inoculation place in order to avoid a neurotoxic paralysis due to the presence of the arthropod salivary glands and the neurotoxin in the patient when the bite is close to a nerve structure[252]. Nevertheless, last American Guidelines for the Prevention, Diagnosis and Treatment of Lyme Disease indicates that if a tick is partially removed, but detached mouthparts remain and cannot easily be removed from the skin, they should be left alone and permitted to fall out[19]. After removing the tick from the skin area, it should be disinfected with povidone iodine, chlorhexidine or other skin disinfectant. Ticks removed should be stored at -20°C for future analyses for the detection or isolation of the causative agent in case of the patient develops an infectious disease[253]. Other popular methods for removing ticks from skin as manual extraction, oil, vaseline, petroleum, lighted cigarettes, among others, are associated with an increase of complications and transmission of infectious agents[246,247]. Taken into account the above considerations, this panel recommends not handling the tick and use forceps for the tick extraction, as is showed in **figure 9** (Consensus level: 9/9).

Figure 9: Recommended type of forceps (A) and tick extraction using forceps (B).



8.2.2. Antibiotic Prophylaxis after Tick Bite

Single-dose doxycycline, given within 72 hours of exposure, is common practice in US and has been evaluated in different clinical studies for postexposure prophylaxis of three spirochetal infections: Lyme disease, syphilis, and tick-borne relapsing fever[254]. The last Guidelines by the Infectious Diseases Society of America (IDSA) recommend prophylactic antibiotic therapy only to adults and children within 72 hours of removal if the tick bite was from an identified *Ixodes* spp., if it occurred in a highly endemic area, and if the tick was attached for ≥36 hours[19]. The preferred antibiotic regimen for the chemoprophylaxis is the administration of a single dose of oral doxycycline (200 mg for adults and 4 mg/kg up to a maximum dose of 200 mg for children) within 72 hours of tick removal over observation. In this case, it has been weighed the likelihood of disease and the effectiveness of prophylactic doxycycline therapy to be higher than the potential risks of the antibiotic. This fact has been not extensible for European countries where it is not recommended with the argument that it will be necessary to perform 40-125 prophylaxes for preventing one borreliosis[255], and the impact on the intestinal flora and a possible development of resistance is conceivable[17]. Anyway, doxycycline prophylaxis must be reconsidered in Europe. Thus, a clinical trial that would allow extending this recommendation to Europe has been recently published[256]. In this open-label, randomized, controlled trial, administering a single dose of 200 mg doxycycline within 72 h after removing an attached tick from the skin, compared to no treatment in people older than eight years resulted in a relative risk reduction of 67% (95% CI 31 - 84%). No serious adverse events were reported. Since we do not have data from Spain and considering that it is a sunny area, a reasonable option might be to consider that when the tick has been manipulated, the tick is engorged or the patient has a high level of anxiety, the prophylaxis with doxycycline could be offered (Consensus level: 9/9).

Safety of doxycycline during pregnancy has not been assessed; therefore, in the case of pregnant women, risks, benefits and uncertainties of doxycycline versus observation should be weighed[254]. In any case, after suffering a tick bite, it is advisable to instruct the patients in the possible signs and symptoms that they may develop and they should at least observe the point of the bite for at least six weeks (Consensus level: 9/9).

CONFLICTS OF INTEREST

1. José A. Oteo declares no conflict of interest in the writing of this article.

2. Héctor Corominas declares no conflict of interest in the writing of this article.

3. Raquel Escudero declares no conflict of interest in the writing of this article.

4. Fernando Fariñas-Guerrero declares no conflict of interest in the writing of this article.

5. Juan Carlos García-Moncó declares no conflict of interest in the writing of this article.

6. Miguel A, Goenaga declares no conflict of interest in the writing of this article.

7. Sara Guillén declares no conflict of interest in the writing of this article.

8. José M. Mascaró declares no conflict of interest in the writing of this article.

9. Aránzazu Portillo declares no conflict of interest in the writing of this article.

REFERENCES

1. Benach JL, Bosler EM, Hanrahan JP, Coleman JL, Habicht GS, Bast TF, et al. Spirochetes isolated from the blood of two patients with Lyme disease. N Engl J Med. 1983; 308(13):740-2. doi: 10.1056/NEJM198303313081302.
2. Steere AC, Grodzicki RL, Kornblatt AN, Craft JE, Barbour AG, Burgdorfer W, et al. The spirochetal etiology of Lyme disease. N Engl J Med. 1983; 308(13):733-40. doi: 10.1056/NEJM198303313081301.
3. Stanek G, Wormser GP, Gray J, Strle F. Lyme borreliosis. Lancet. 2012; 379(9814):461-73. doi: 10.1016/S0140-6736(11)60103-7.
4. Steere AC, Malawista SE, Hardin JA, Ruddy S, Askenase W, Andiman WA. Erythema chronicum migrans and Lyme arthritis. The enlarging clinical spectrum. Ann Intern Med. 1977; 86(6):685-98. doi: 10.7326/0003-4819-86-6-685.
5. Steere AC, Malawista SE, Snydman DR, Shope RE, Andiman WA, Ross MR, et al. Lyme arthritis: an epidemic of oligoarticular arthritis in children and adults in three Connecticut communities. Arthritis Rheum. 1977; 20(1):7-17. doi: 10.1002/art.1780200102.
6. Burgdorfer W, Barbour AG, Hayes SF, Benach JL, Grunwaldt E, Davis JP. Lyme disease-a tick-borne spirochetosis? Science. 1982; 216(4552):1317-9. doi: 10.1126/science.7043737.
7. https://www.ecdc.europa.eu/sites/default/files/media/en/healthtopics/emerging_and_vector-borne_diseases/tick_borne_diseases/public_health_measures/Documents/HCP_factsheet_LB_highres.pdf
8. Stanek G, Fingerle V, Hunfeld KP, Jaulhac B, Kaiser R, Krause A, et al. Lyme borreliosis: clinical case definitions for diagnosis and management in Europe. Clin Microbiol Infect. 2011; 17(1):69-79. doi: 10.1111/j.1469-0691.2010.03175.x.
9. Oteo JA. Meningitis aséptica aguda: muchas causas a considerar [Acute aseptic meningitis. Many causes to consider]. Enferm Infecc Microbiol Clin. 2012; 30(7):359-60. doi: 10.1016/j.eimc.2012.05.004.
10. Brouqui P, Bacellar F, Baranton G, Birtles RJ, Bjoërsdorff A, Blanco JR, et al. Guidelines for the diagnosis of tick-borne bacterial diseases in Europe. Clin Microbiol Infect. 2004; 10(12):1108-32. doi: 10.1111/j.1469-0691.2004.01019.x.
11. Moore A, Nelson C, Molins C, Mead P, Schriefer M. Current Guidelines, Common Clinical Pitfalls, and Future Directions for Laboratory Diagnosis of Lyme Disease, United States. Emerg Infect Dis. 2016; 22(7):1169–77. doi: 10.3201/eid2207.151694.
12. Cutler SJ, Rudenko N, Golovchenko M, Cramaro WJ, Kirpach J, Savic S, et al. Diagnosing Borreliosis. Vector Borne Zoonotic Dis. 2017; 17(1):2-11. doi: 10.1089/vbz.2016.1962.
13. Dessau RB, van Dam AP, Fingerle V, Gray J, Hovius JW, Hunfeld KP, et al. To test or not to test? Laboratory support for the diagnosis of Lyme borreliosis: a position paper of ESGBOR, the ESCMID study group for Lyme borreliosis. Clin Microbiol Infect. 2018; 24(2):118-124. doi: 10.1016/j.cmi.2017.08.025.
14. Figoni J, Chirouze C, Hansmann Y, Lemogne C, Hentgen V, Saunier A, et al. Lyme borreliosis and other tick-borne diseases. Guidelines from the French Scientific Societies (I): prevention, epidemiology, diagnosis. Med Mal Infect. 2019; 49(5):318-334. doi:

10.1016/j.medmal.2019.04.381.
15. Jaulhac B, Saunier A, Caumes E, Bouiller K, Gehanno JF, Rabaud C, et al. Lyme borreliosis and other tick-borne diseases. Guidelines from the French scientific societies (II). Biological diagnosis, treatment, persistent symptoms after documented or suspected Lyme borreliosis. Med Mal Infect. 2019; 49(5):335-346. doi: 10.1016/j.medmal.2019.05.001.
16. Mead P, Petersen J, Hinckley A. Updated CDC Recommendation for Serologic Diagnosis of Lyme Disease. MMWR Morb Mortal Wkly Rep. 2019; 68(32):703. doi: 10.15585/mmwr.mm6832a4.
17. Rauer S, Kastenbauer S, Hofmann H, Fingerle V, Huppertz HI, Hunfeld KP, et al. Guidelines for diagnosis and treatment in neurology - Lyme neuroborreliosis. Ger Med Sci. 2020; 18:Doc03. doi: 10.3205/000279.
18. Talagrand-Reboul E, Raffetin A, Zachary P, Jaulhac B, Eldin C. Immunoserological Diagnosis of Human Borrelioses: Current Knowledge and Perspectives. Front Cell Infect Microbiol. 2020; 10:241. doi: 10.3389/fcimb.2020.00241.
19. Lantos PM, Rumbaugh J, Bockenstedt LK, Falck-Ytter YT, Aguero-Rosenfeld ME, Auwaerter PG, et al. Clinical Practice Guidelines by the Infectious Diseases Society of America (IDSA), American Academy of Neurology (AAN), and American College of Rheumatology (ACR): 2020 Guidelines for the Prevention, Diagnosis, and Treatment of Lyme Disease. Arthritis Care Res (Hoboken). 2021; 73(1):1-9. doi: 10.1002/acr.24495.
20. Portillo A, Santibáñez S, Oteo JA. Enfermedad de Lyme [Lyme disease]. Enferm Infecc Microbiol Clin. 2014;32 Suppl 1:37-42. doi: 10.1016/S0213-005X(14)70148-X.
21. Raffetin A, Saunier A, Bouiller K, Caraux-Paz P, Eldin C, Gallien S, et al. Unconventional diagnostic tests for Lyme borreliosis: a systematic review. Clin Microbiol Infect. 2020; 26(1):51-59. doi: 10.1016/j.cmi.2019.06.033.
22. Oteo JA, Martinez de Artola V, Casas J, Lozano A, Fernandez Calvo JL, Grandival R. Epidemiology and prevalence of seropositivity against *Borrelia burgdorferi* antigen in La Rioja, Spain. Rev Epidemiol Sante Publique. 1992; 40(2):85-92. PMID: 1631381.
23. Dattwyler RJ, Volkman DJ, Luft BJ, Halperin JJ, Thomas J, Golightly MG. Seronegative Lyme disease. Dissociation of specific T- and B-lymphocyte responses to *Borrelia burgdorferi*. N Engl J Med. 1988; 319(22):1441-6. doi: 10.1056/NEJM198812013192203.
24. Rudenko N, Golovchenko M, Vancova M, Clark K, Grubhoffer L, Oliver JH Jr. Isolation of live *Borrelia burgdorferi* sensu lato spirochaetes from patients with undefined disorders and symptoms not typical for Lyme borreliosis. Clin Microbiol Infect. 2016; 22(3):267.e9-15. doi: 10.1016/j.cmi.2015.11.009.
25. Wang G, Schwartz I. Genus *Borrelia*. In: Krieg NRS, Staley JT, Brown DR, Hedlund BP, Paster BJ, Ward NL, Ludwig W, Whitman WB, editors. Bergey's Manual of Systematic Bacteriology, Vol. 4: The *Bacteroidetes, Spirochaetes, Tenericutes (Mollicutes), Acidobacteria, Fibrobacteres, Fusobacteria, Dictyoglomi, Gemmatimonadetes, Lentisphaerae, Verrucomicrobia, Chlamydiae,* and *Planctomycetes.*2. Springer; New York: 2011. pp. 484–531.
26. Pritt BS, Mead PS, Johnson DKH, Neitzel DF, Respicio-Kingry LB, Davis JP, et al. Identification of a novel pathogenic *Borrelia* species causing Lyme borreliosis with unusually high spirochaetaemia: a descriptive study. Lancet Infect Dis. 2016; 16(5):556-564. doi: 10.1016/S1473-3099(15)00464-8.
27. Adeolu M, Gupta RS. A phylogenomic and molecular marker based proposal for the division of the genus *Borrelia* into two genera: the emended genus *Borrelia* containing only the members of the relapsing fever *Borrelia*, and the genus *Borreliella* gen. nov. containing the members of the Lyme disease *Borrelia* (*Borrelia burgdorferi* sensu lato complex). Antonie Van Leeuwenhoek. 2014;105(6):1049-72. doi: 10.1007/s10482-014-0164-x.
28. Margos G, Castillo-Ramirez S, Cutler S, Dessau RB, Eikeland R, Estrada-Peña A, et al. Rejection of the name *Borreliella* and all proposed species comb. nov. placed therein. Int J Syst Evol Microbiol. 2020; 70(5):3577-3581. doi: 10.1099/ijsem.0.004149.
29. Uría DF, Calatayud M, Mongelos JM, Miguel MD, Cobos A, Suárez T. Meningopolineuritis como manifestación de la enfermedad de Lyme [Meningopolyneuritis as a manifestation of Lyme disease]. Med Clin (Barc). 1987; 89(9):381-3. PMID: 3669789.
30. Rodríguez Torres A, Miranda A, Quiñones PA, Pérez Oliva N, Bratos MA, Orduña A, et al. Eritema crónico migratorio por *Borrelia burgdorferi* [Erythema chronicum migrans caused by *Borrelia burgdorferi*]. Med Clin (Barc). 1988; 91(8):297-9. Erratum in: Med Clin (Barc) 1988; 91(15):569. PMID: 3251480.
31. Maraví Petri E, Oteo Revuelta JA, Pérez Gómez JM, De Miguel Medina C, López Unzu A. Meningoradiculitis linfocitaria (síndrome de Bannwarth). Expresión neurológica de la enfermedad de Lyme [Lymphocytic meningoradiculitis (Bannwarth's syndrome). Neurologic involvement of

Lyme disease]. Rev Med Univ Navarra. 1989; 33(1):19-22. PMID: 2490177.
32. Artigao R, Torres G, Guerrero A, Jiménez-Mena M, Bayas Paredes M. Irreversible complete heart block in Lyme disease. Am J Med. 1991; 90(4):531-3. PMID: 2012098.
33. España A, Torrelo A, Guerrero A, Suárez J, Rocamora A, Ledo A. Periarticular fibrous nodules in Lyme borreliosis. Br J Dermatol. 1991; 125(1):68-70. doi: 10.1111/j.1365-2133.1991.tb06043.x.
34. Anda P, Rodríguez I, de la Loma A, Fernández MV, Lozano A. A serological survey and review of clinical Lyme borreliosis in Spain. Clin Infect Dis. 1993; 16(2):310-9. doi: 10.1093/clind/16.2.310.
35. García-Moncó JC, Gómez Beldarrain M, Estrade L. Painful lumbosacral plexitis with increased ESR and *Borrelia burgdorferi* infection. Neurology. 1993; 43(6):1269. doi: 10.1212/wnl.43.6.1269.
36. Guerrero A, Escudero R, Martí-Belda P, Quereda C, Miramón J. Lyme borreliosis in Spain: a serological survey. Clin Infect Dis. 1994; 18(3):493-4. doi: 10.1093/clinids/18.3.493.
37. Guerrero A, Serrano MJ. Frecuencia y espectro clínico de la infección por *Borrelia burgdorferi* en España. Grupo de Estudio para la Enfermedad de Lyme en España [Incidence and clinical spectrum of *Borrelia burgdorferi* infection in Spain. Study group for Lyme disease in Spain]. Med Clin (Barc). 1989; 92(11):438-9. PMID: 2786124.
38. Oteo Revuelta JA, Martínez de Artola V, Gómez-Cadiñanos R, Casas Fernández-Tejerina JM, Grandival García R. Eritema crónico migratorio (enfermedad de Lyme). Estudio clínico epidemiológico de diez casos [Erythema chronicum migrans (Lyme's disease). Clinico-epidemiologic study of 10 cases]. Rev Clin Esp. 1993; 193(1):20-3. PMID: 8337455.
39. Oteo Revuelta JA, Blanco Ramos JR, Martínez de Artola V, Grandival García R, Ibarra Cucalón V, Dopereiro Gómez R. Eritema migratorio (borreliosis de Lyme). Características clinicoepidemiológicas de 50 pacientes [Migratory erythema (Lyme borreliosis). Clinicoepidemiologic features of 50 patients]. Rev Clin Esp. 2000; 200(2):60-3. doi: 10.1016/s0014-2565(00)70564-9.
40. Gómez-Eguílaz M, Gómez-Cerquera J, Calvo-Pérez L, Oteo JA. Neuroborreliosis: A single-hospital series of 7 cases. Neurologia. 2016; 31(2):137-9. doi: 10.1016/j.nrl.2014.03.008.
41. Oteo JA, Martínez de Artola V, Fernández Calvo JL, Casas JM, Rivero A, Grandival R. Prevalencia de anticuerpos frente a *Borrelia burgdorferi* en una población de riesgo [The prevalence of *Borrelia burgdorferi* antibodies in a population at risk]. Rev Clin Esp. 1990; 187(5):215-7. PMID: 2102530.
42. Oteo Revuelta JA, Elías Calvo C, Martínez de Artola V, Pérez Surribas D. Infección por *Borrelia burgdorferi* en pacientes infectados por el virus de la inmunodeficiencia humana. Un problema diagnóstico [Infection by *Borrelia burgdorferi* in patients with the human immunodeficiency virus. A diagnostic problem]. Med Clin (Barc). 1993; 101(6):207-9. PMID: 8332020.
43. Saz JV, Nuncio S, Merino FJ, Aquise M, Medina J, Filipe AR. Enfermedad de Lyme en la provincia de Soria: estudio clinicoepidemiológico [Lyme disease in the province of Soria: clinico-epidemiologic study]. Enferm Infecc Microbiol Clin. 1994; 12(2):52-9. PMID: 8011711.
44. Gutierrez J, Maroto MC, De la Higuera A, Guerrero M, Padilla E, Piédrola G. Three-year study of antibody to *Borrelia burgdorferi* in southern Spain. Eur J Clin Microbiol Infect Dis. 1995; 14(6):542-6. doi: 10.1007/BF02113437.
45. Rojo Vázquez J. Seroprevalencia de la infección por *Borrelia burgdorgferi* y *Rickettsia conorii* en población humana y canina de La Zona Básica de Salud de San Andrés del Rabanedo (León, España) [Seroprevalence of the infections caused by *Borrelia burgdorferi* and *Rickettsia conorii* in humans and dogs in primary health care of San Andres del Rabanedo (Leon, Spain)]. Rev Esp Salud Publica. 1997; 71(2):173-80. PMID: 9546860.
46. Arteaga F, Golightly MG, Garcia Perez A, Barral M, Anda P, Garcia-Monco JC. Disparity between serological reactivity to *Borrelia burgdorferi* and evidence of past disease in a high-risk group. Clin Infect Dis. 1998; 27(5):1210-3. doi: 10.1086/514970.
47. Lledó L, Gegúndez MI, Saz JV, Beltrán M. Screening of the prevalence of antibodies to *Borrelia burgdorferi* in Madrid province, Spain. Eur J Epidemiol. 2004; 19(5):471-2. doi: 10.1023/b:ejep.0000027349.48337.cb.
48. Segura F, Diestre G, Sanfeliu I, Cardeñosa N. Seroprevalencia de la infección por *Borrelia burgdorferi* en el área del Vallès Occidental (Barcelona) [Seroprevalence of *Borrelia burgdorferi* infection in the area of Valles Occidental (Barcelona, Spain)]. Med Clin (Barc). 2004; 123(10):395. doi: 10.1016/s0025-7753(04)74527-3.
49. Alcalde-Encinas MD, Peñalver-González E, Pavia AR, Tornel Sánchez G. Utilidad diagnóstica de la serología a *B. burgdorferi* en Cartagena [Diagnostic utility of serology to *B. burgdorferi* in Cartagena]. Rev Clin Esp. 2010; 210(6):314. doi: 10.1016/j.rce.2009.10.006. Erratum in: Rev Clin Esp. 2010; 210(11):597. García, G Tornel [corrected to Tornel Sánchez, G]. PMID: 20434146.
50. Oteiza-Olaso J, Tiberio-López G, Martínez de Artola V, Belzunegui-Otano T. Seroprevalencia de

la enfermedad de Lyme en Navarra [Seroprevalence of Lyme disease in Navarra, Spain]. Med Clin (Barc). 2011; 136(8):336-9. doi: 10.1016/j.medcli.2010.06.008.
51. Lledó L, Gegúndez MI, Giménez-Pardo C, Álamo R, Fernández-Soto P, Nuncio MS, et al. A seventeen-year epidemiological surveillance study of *Borrelia burgdorferi* infections in two provinces of northern Spain. Int J Environ Res Public Health. 2014; 11(2):1661-72. doi: 10.3390/ijerph110201661.
52. Barreiro-Hurlé L, Melón-García S, Seco-Bernal C, Muñoz-Turrillas C, Rodríguez-Pérez M. Seroprevalence of Lyme disease in southwest Asturias. Enferm Infecc Microbiol Clin. 2020; 38(4):155-158. doi: 10.1016/j.eimc.2019.06.010.
53. Lledó L, Giménez-Pardo C, Gegúndez MI. Screening of forestry workers in Guadalajara Province (Spain) for antibodies to Lymphocytic Choriomeningitis Virus, Hantavirus, *Rickettsia* spp. and *Borrelia burgdorferi*. Int J Environ Res Public Health. 2019; 16(22):4500. doi: 10.3390/ijerph16224500.
54. Lindgren E, Jaenson TG: Lyme Borreliosis in Europe: Influences of Climate and Climate Change, Epidemiology, Ecology, and Adaptation Measures. Geneva, World Health Organization, 2006.
55. Hofmann H, Fingerle V, Hunfeld KP, Huppertz HI, Krause A, Rauer S, et al. Cutaneous Lyme borreliosis: Guideline of the German Dermatology Society. Ger Med Sci. 2017; 15:Doc14. doi: 10.3205/000255.
56. Oteo Revuelta JA, Estrada Peña A. *Ixodes ricinus*, vector comprobado de *Borrelia burgdorferi* en España [*Ixodes ricinus*, a demonstrated vector of *Borrelia burgdorferi* in Spain]. Med Clin (Barc). 1991; 96(15):599. PMID: 2051824.
57. Estrada-Peña A, Oteo JA, Estrada-Peña R, Gortázar C, Osácar JJ, Moreno JA, et al. *Borrelia burgdorferi* sensu lato in ticks (Acari: Ixodidae) from two different foci in Spain. Exp Appl Acarol. 1995; 19(3):173-80. doi: 10.1007/BF00046289.
58. Barral M, García-Pérez AL, Juste RA, Hurtado A, Escudero R, Sellek RE, et al. Distribution of *Borrelia burgdorferi* sensu lato in *Ixodes ricinus* (Acari: *Ixodidae*) ticks from the Basque Country, Spain. J Med Entomol. 2002; 39(1):177-84. doi: 10.1603/0022-2585-39.1.177.
59. Fernández-Soto, P. Garrapatas que parasitan a las personas en Castilla y León, determinación por serología de su parasitismo y detección molecular de los patógenos que albergan. Tesis doctoral. Universidad de Salamanca; 2003.
60. Gil H, Barral M, Escudero R, García-Pérez AL, Anda P. Identification of a new *Borrelia* species among small mammals in areas of northern Spain where Lyme disease is endemic. Appl Environ Microbiol. 2005; 71(3):1336-45. doi: 10.1128/AEM.71.3.1336-1345.2005.
61. Estrada-Peña A, Osácar JJ, Pichon B, Gray JS. Hosts and pathogen detection for immature stages of *Ixodes ricinus* (Acari: Ixodidae) in North-Central Spain. Exp Appl Acarol. 2005; 37(3-4):257-68. doi: 10.1007/s10493-005-3271-6.
62. Barandika JF, Hurtado A, García-Sanmartín J, Juste RA, Anda P, García-Pérez AL. Prevalence of tick-borne zoonotic bacteria in questing adult ticks from northern Spain. Vector Borne Zoonotic Dis. 2008; 8(6):829-35. doi: 10.1089/vbz.2008.0023.
63. Ruiz-Fons F, Fernández-de-Mera IG, Acevedo P, Gortázar C, de la Fuente J. Factors driving the abundance of *Ixodes ricinus* ticks and the prevalence of zoonotic *I. ricinus*-borne pathogens in natural foci. Appl Environ Microbiol. 2012; 78(8):2669-76. doi: 10.1128/AEM.06564-11.
64. Palomar AM, Santibáñez P, Mazuelas D, Roncero L, Santibáñez S, Portillo A, Oteo JA. Role of birds in dispersal of etiologic agents of tick-borne zoonoses, Spain, 2009. Emerg Infect Dis. 2012; 18(7):1188-91. doi: 10.3201/eid1807.111777.
65. Palomar AM, Portillo A, Santibáñez P, Mazuelas D, Roncero L, Gutiérrez Ó, et al. Presence of *Borrelia turdi* and *Borrelia valaisiana* (Spirochaetales: Spirochaetaceae) in Ticks Removed From Birds in the North of Spain, 2009-2011. J Med Entomol. 2017;54(1):243-246. doi: 10.1093/jme/tjw158.
66. Palomar AM, Portillo A, Santibáñez P, Santibáñez S, Oteo JA. *Borrelia miyamotoi*: Should this pathogen be considered for the diagnosis of tick-borne infectious diseases in Spain? Enferm Infecc Microbiol Clin (Engl Ed). 2018; 36(9):568-571. doi: 10.1016/j.eimc.2017.10.020.
67. Espí A, Del Cerro A, Somoano A, García V, M Prieto J, Barandika JF, et al. *Borrelia burgdorferi* sensu lato prevalence and diversity in ticks and small mammals in a Lyme borreliosis endemic Nature Reserve in North-Western Spain. Incidence in surrounding human populations. Enferm Infecc Microbiol Clin. 2017; 35(9):563-568. doi: 10.1016/j.eimc.2016.06.011.
68. Estrada-Peña A, Roura X, Sainz A, Miró G, Solano-Gallego L. Species of ticks and carried pathogens in owned dogs in Spain: Results of a one-year national survey. Ticks Tick Borne Dis. 2017; 8(4):443-452. doi: 10.1016/j.ttbdis.2017.02.001.

69. Díaz P, Arnal JL, Remesar S, Pérez-Creo A, Venzal JM, Vázquez-López ME, et al. Molecular identification of *Borrelia* spirochetes in questing *Ixodes ricinus* from northwestern Spain. Parasit Vectors. 2017; 10(1):615. doi: 10.1186/s13071-017-2574-x.
70. Díaz P, Remesar S, Venzal JM, Vázquez-López ME, Fernández G, López C, et al. Occurrence of *Borrelia* and *Borreliella* species in *Ixodes ricinus* collected from roe deer in northwestern Spain. Med Vet Entomol. 2019; 33(3):427-430. doi: 10.1111/mve.12364.
71. Remesar S, Díaz P, Venzal JM, Prieto A, Estrada-Peña A, López CM, et al. Longitudinal Study of Infection with *Borrelia* spp. in Questing Ticks from North-Western Spain. Vector Borne Zoonotic Dis. 2019; 19(11):785-792. doi: 10.1089/vbz.2019.2442.
72. García-Moncó JC, Benach JL, Coleman JL, Galbe JL, Szczepanski A, Fernández Villar B, et al. Caracterización de una cepa española de *Borrelia burgdorferi* [The characterization of a Spanish strain of *Borrelia burgdorferi*]. Med Clin (Barc). 1992; 98(3):89-93. PMID: 1552756.
73. Escudero R, Barral M, Pérez A, Vitutia MM, García-Pérez AL, Jiménez S, et al. Molecular and pathogenic characterization of *Borrelia burgdorferi* sensu lato isolates from Spain. J Clin Microbiol. 2000; 38(11):4026-33. doi: 10.1128/JCM.38.11.4026-4033.2000.
74. Oteo JA, Backenson PB, del Mar Vitutia M, García Moncó JC, Rodríguez I, Escudero R, et al. Use of the C3H/He Lyme disease mouse model for the recovery of a Spanish isolate of *Borrelia garinii* from erythema migrans lesions. Res Microbiol. 1998; 149(1):39-46. doi: 10.1016/s0923-2508(97)83622-4.
75. Oteo JA, Guerrero A. Propuesta de definición de zona endémica de borreliosis de Lyme. Enf Infecc Microbiol Clin. 1996; 14:517.
76. Alonso Vigil P, Rodríguez Suárez L, Margolles Martins M, Servicio de Vigilancia y Alertas Epidemiológicas. Borreliosis de Lyme. Características clínico-epidemiológicas de la infección en el Principado de Asturias. Edición: Consejería de Sanidad. Dirección General de Salud Pública. Servicio de Vigilancia y Alertas Epidemiológicas. 2015. Disponible en: http://www.astursalud.es (estadísticas y epidemiología).
77. Vázquez-López ME, Pego-Reigosa R, Diez-Morrondo C, Castro-Gago M, Díaz P, Fernández G, et al. Epidemiology of Lyme disease in a healthcare area in North-West Spain. Gac Sanit. 2015; 29:213–216. doi: 10.1016/j.gaceta.2015.01.008.
78. Treviño Castellano M, Navarro de la Cruz D, Trastoy Pena R. Serología de *Borrelia burgdorferi* y diagnóstico de la enfermedad de Lyme en el área sanitaria de Santiago de Compostela (Galicia): 2006-2016. Med Clin (Barc). 2018; 151:162–163.doi: 10.1016/j.medcli.2017.11.013
79. Palomar AM. Papel de las aves en la dispersión de garrapatas y sus microorganismos. 2017. (Tesis Doctoral). Disponible en: https://dialnet.unirioja.es/descarga/tesis/122702.pdf
80. Portillo A, Palomar AM, de Toro M, Santibáñez S, Santibáñez P, Oteo JA. Exploring the bacteriome in anthropophilic ticks: To investigate the vectors for diagnosis. PLoS One. 2019;14(3):e0213384. doi: 10.1371/journal.pone.0213384.
81. Jado I, Oteo JA, Aldámiz M, Gil H, Escudero R, Ibarra V, et al. *Rickettsia monacensis* and human disease, Spain. Emerg Infect Dis. 2007; 13(9):1405-1407. doi: 10.3201/eid1309.060186.
82. Fernández-Soto P, Pérez-Sánchez R, Encinas-Grandes A, Sanz RA. Detection and identification of *Rickettsia helvetica* and *Rickettsia* sp. IRS3/IRS4 in *Ixodes ricinus* ticks found on humans in Spain. Eur J Clin Microbiol Infect Dis. 2004;23(8):648-649). doi: 10.1007/s10096-004-1184-7.
83. Oteo JA, Blanco JR, Martínez de Artola V, Ibarra V. First report of human granulocytic ehrlichiosis from southern Europe (Spain) Emerg Infect Dis. 2000; 6(4):430-432. doi: 10.3201/eid0604.000425.
84. Portillo A, Pérez-Martínez L, Santibáñez S, Santibáñez P, Palomar AM, Oteo JA. *Anaplasma s*pp. in wild mammals and *Ixodes ricinus* from the north of Spain. Vector Borne Zoonotic Dis. 2011;11(1):3-8. doi: 10.1089/vbz.2009.0214.
85. Palomar AM, García-Álvarez L, Santibáñez S, Portillo A, Oteo JA. Detection of tick-borne '*Candidatus* Neoehrlichia mikurensis' and *Anaplasma phagocytophilum* in Spain in 2013. Parasit Vectors. 2014; 7:57. doi: 10.1186/1756-3305-7-57.
86. Oteo JA, Estrada A. Human babesiosis. Study of vectors. Enferm Infecc Microbiol Clin. 1992; 10(8):466-469.
87. Miguélez M, Linares Feria M, González A, Mesa MC, Armas F, Laynez P. Babesiosis humana en un paciente esplenectomizado [Human babesiosis in a patient after splenectomy]. Med Clin (Barc). 1996;106(11):427-429.
88. Palomar AM, Portillo A, Santibáñez P, Santibáñez S, García-Álvarez L, Oteo JA. Genetic characterization of *Candidatus* Rickettsia vini, a new rickettsia amplified in ticks from La Rioja, Spain. Ticks Tick Borne Dis. 2012; 3(5-6): 319-321. doi: 10.1016/j.ttbdis.2012.10.025.
89. Barandika JF, Hurtado A, Juste RA, García-Pérez AL. Seasonal dynamics of *Ixodes ricinus* in a 3-

year period in northern Spain: first survey on the presence of tick-borne encephalitis virus. Vector Borne Zoonotic Dis. 2010; 10(10):1027-1035. doi:10.1089/vbz.2009.0148
90. Palomar AM, Portillo A, Eiros JM, Oteo JA. The risk of introducing tick-borne encephalitis and Crimean-Congo hemorrhagic fever into Southwestern Europe (Iberian Peninsula). In: Virology II – advanced issues 2013. ISBN: 978-1-922227-45-4. Available from: https://www.iconceptpress.com/book/virology-ii–advanced-issues/11000086/1206000594/
91. Oteo-Revuelta JA, Martínez de Artola V. Lyme borreliosis: epidemiologic and etiopathogenic aspects. Enferm Infecc Microbiol Clin. 1995; 13(9):550-555.
92. Palomar AM, Portillo A, Santibañez S, San José C, Rayas E, Talavera V, et al. Distribución y prevalencia de microorganismos trasmitidos por garrapatas en el espacio natural Doñana (Huelva) y en el Parque Natural Los Alcornocales (Cádiz). XXIII Congreso Nacional de la Sociedad Española de Enfermedades Infecciosas y Microbiología Clínica. Madrid 23-25 de mayo de 2019.
93. Jore S, Viljugrein H, Hofshagen M, Brun-Hansen H, Kristoffersen AB, Nygård K, et al. Multi-source analysis reveals latitudinal and altitudinal shifts in range of *Ixodes ricinus* at its northern distribution limit. Parasit Vectors. 2011; 1:84. doi: 10.1186/1756-3305-4-84.
94. Medlock JM, Hansford KM, Bormane A, Derdakova M, Estrada-Peña A, George JC, et al. Driving forces for changes in geographical distribution of *Ixodes ricinus* ticks in Europe. Parasit Vectors. 2013; 6:1. doi: 10.1186/1756-3305-6-1.
95. Garcia-Vozmediano A, Krawczyk AI, Sprong H, Rossi L, Ramassa E, Tomassone L. Ticks climb the mountains: Ixodid tick infestation and infection by tick-borne pathogens in the Western Alps. Ticks Tick Borne Dis. 2020; 11(5):101489. doi: 10.1016/j.ttbdis.2020.101489.
96. Fernández-Ruiz N, Estrada-Peña A. Could climate trends disrupt the contact rates between *Ixodes ricinus* (Acari, Ixodidae) and the reservoirs of *Borrelia burgdorferi* s.l.? PLoS One. 2020; 15(5):e0233771. doi: 10.1371/journal.pone.0233771.
97. Estrada-Peña A, Martinez JM, Acedo CS, Quilez J, Del Cacho E. Phenology of the tick, *Ixodes ricinus*, in its southern distribution range (central Spain). Med Vet Entomol. 2004; 18(4):387-97.doi: 10.1111/j.0269-283X.2004.00523.x.
98. Remesar S, Fernández PD, Venzal JM, Pérez-Creo A, Prieto A, Estrada-Peña A, et al. Tick species diversity and population dynamics of *Ixodes ricinus* in Galicia (north-western Spain). Ticks Tick Borne Dis. 2019; 10(1):132-137. doi: 10.1016/j.ttbdis.2018.09.006.
99. Buchwald A. Ein Fall von diffuser idiopathischer Haut-Atrophie. Arch Derm Res. 1883; 10:553–556. doi: 10.1007/BF01833474.
100. Afzelius A. Verhandlungen der dermatologischen gesellschaff zu Stockholm sitzung vom 28. Oktober 1909. Archiv fur Dermatologie and Syphilis 1910; 101:404
101. Bäfverstedt B. Lymphadenosis benigna cutis (LABC). Its nature, course and prognosis. Acta Derm Vewnereol (Stockh) 1960; 40:10-18.
102. Steere AC. Lyme disease. N Engl J Med. 2001; 345:115-25. doi: 10.1056/NEJ M200107123450207.
103. Stanek G, Strle F. Lyme disease: European perspective. Infect Dis Clin North Am. 2008; 22:327-39, vii. doi: 10.1016/j.idc.2008.01.001.
104. Glatz M, Resinger A, Semmelweis K, Ambros-Rudolph CM, Müllegger RR. Clinical spectrum of skin manifestations of Lyme borreliosis in 204 children in Austria. Acta Derm Venereol. 2015; 95(5):565-71. doi: 10.2340/00015555-2000.
105. Oteo JA, Ibarra V. DEBONEL (*Dermacentor*-borne-necrosis-erythema-lymphadenopathy). ¿Una nueva enfermedad transmitida por garrapatas? [DEBONEL (*Dermacentor*-borne-necrosis-erythemalymphadenopathy). A new tick-borne disease?]. Enferm Infecc Microbiol Clin. 2002; 20(2):51-2. PMID: 11886671.
106. Campbell GL, Paul WS, Schriefer ME, Craven RB, Robbins KE, Dennis DT. Epidemiologic and diagnostic studies of patients with suspected early Lyme disease, Missouri, 1990-1993. J Infect Dis. 1995; 172(2):470-80. doi: 10.1093/infdis/172.2.470. PMID: 7622891.
107. Oteo JA. Linfadenosis benigna cutis. Aportación de dos casos. VI Reunión Nacional de Enfermedades Infecciosas y Microbiología Clínica. Sitges, 19-20 octubre 1995.
108. Ogrinc K, Maraspin V, Lusa L, Cerar Kišek T, Ružić-Sabljić E, Strle F. Acrodermatitis chronica atrophicans: clinical and microbiological characteristics of a cohort of 693 Slovenian patients. J Intern Med. 2021. doi: 10.1111/joim.13266.
109. Moniuszko-Malinowska A, Czupryna P, Dunaj J, Pancewicz S, Garkowski A, Kondrusik M, et al. Acrodermatitis chronica atrophicans: various faces of the late form of Lyme borreliosis. Postepy Dermatol Alergol. 2018; 35(5):490-494. doi: 10.5114/ada.2018.77240.
110. Garcia-Monco JC, Benach JL. Lyme neuroborreliosis. Ann Neurol. 1995; 37(6):691-702. doi:

10.1002/ana.410370602.
111. Halperin JJ, Garcia-Monco JC. The Human Borreliosis: Lyme neuroborreliosis and Relapsing Fever. In: Garcia-Monco JC, editor. CNS Infections. A Clinical Approach. London: Springer; 2017.
112. Garcia-Monco JC, Benach JL. Lyme Neuroborreliosis: Clinical Outcomes, Controversy, Pathogenesis, and Polymicrobial Infections. Ann Neurol. 2019; 85(1):21-31. doi: 10.1002/ana.25389.
113. Knudtzen FC, Andersen NS, Jensen TG, Skarphedinsson S. Characteristics and Clinical Outcome of Lyme Neuroborreliosis in a High Endemic Area, 1995-2014: A Retrospective Cohort Study in Denmark. Clin Infect Dis. 2017; 65(9):1489-95. doi: 10.1093/cid/cix568.
114. Schwenkenbecher P, Pul R, Wurster U, Conzen J, Pars K, Hartmann H, et al. Common and uncommon neurological manifestations of neuroborreliosis leading to hospitalization. BMC Infect Dis. 2017; 17(1):90. doi: 10.1186/s12879-016-2112-z.
115. Schwartz AM, Hinckley AF, Mead PS, Hook SA, Kugeler KJ. Surveillance for Lyme Disease - United States, 2008-2015. MMWR Surveill Summ. 2017;66(22):1-12. doi: 10.15585/mmwr.ss6622a1.
116. Kruger H, Kohlhepp W, Konig S. Follow-up of antibiotically treated and untreated neuroborreliosis. Acta Neurol Scand. 1990; 82(1):59-67. doi: 10.1111/j.1600-0404.1990.tb01588.x.
117. Pachner AR, Steere AC. The triad of neurologic manifestations of Lyme disease: meningitis, cranial neuritis, and radiculoneuritis. Neurology. 1985; 35(1):47-53. doi: 10.1212/wnl.35.1.47.
118. Ackermann R, Gollmer E, Rehse-Kupper B. [Progressive *Borrelia* encephalomyelitis. Chronic manifestation of erythema chronicum migrans disease of the nervous system]. Dtsch Med Wochenschr. 1985; 110(26):1039-42. doi: 10.1055/s-2008-1068956.
119. Kohler J, Kasper J, Kern U, Thoden U, Rehse-Kupper B. Borrelia encephalomyelitis. Lancet. 1986; 2(8497):35. doi: 10.1016/s0140-6736(86)92574-2.
120. Kruger H, Reuss K, Pulz M, Rohrbach E, Pflughaupt KW, Martin R, et al. Meningoradiculitis and encephalomyelitis due to *Borrelia burgdorferi*: a follow-up study of 72 patients over 27 years. J Neurol. 1989; 236(6):322-8. doi: 10.1007/BF00314373.
121. Huda S, Wieshmann UC. Protracted neuroborreliosis-an unusual cause of encephalomyelitis. BMJ Case Rep. 2012; 2012:bcr1120115206. doi: 10.1136/bcr.11.2011.5206.
122. Beuchat I, Dunet V, Meylan P, Du Pasquier R. Late Lyme neuroborreliosis with chronic encephalomyelitis. Neurology. 2018; 91(13):627-8. doi: 10.1212/WNL.0000000000006252.
123. Mygland A, Ljostad U, Fingerle V, Rupprecht T, Schmutzhard E, Steiner I, et al. EFNS guidelines on the diagnosis and management of European Lyme neuroborreliosis. Eur J Neurol. 2010; 17(1):8-16, e1-4. doi: 10.1111/j.1468-1331.2009.02862.x.
124. Bonet-Alaves E, Guerrero-Espejo A, Cuenca Torres M, Gimeno Vilarrasa F. Incidencia de la enfermedad de Lyme en España. Med Clin (Barc). 2016; 147:88–9. doi: 10.1016/j.medcli.2016.01.021.
125. Lobo-Prat D, Corominas H, Pomar V. Suitability of serology tests for the diagnosis of Lyme disease. Single center urban cohort. Med Clin (Barc). 2020; 2:S0025-7753(20)30733-8. doi: 10.1016/j.medcli.2020.08.010.
126. Dennis DT, Hayes EB. Epidemiology of Lyme Borreliosis. In: Lyme borreliosis: biology, epidemiology and control, Kahl O, Gray JS, Lane RS, Stanek G (Eds), CABI Publishing, Oxford 2002. p.251.
127. Grillon A, Scherlinger M, Boyer PH, De Martino S, Perdriger A, Blasquez A, et al. Characteristics and clinical outcomes after treatment of a national cohort of PCR-positive Lyme arthritis. Semin Arthritis Rheum. 2019; 48(6):1105-1112. doi: 10.1016/j.semarthrit.2018.09.007.
128. Brouwer MAE, van de Schoor FR, Vrijmoeth HD, Netea MG, Joosten LAB. A joint effort: The interplay between the innate and the adaptive immune system in Lyme arthritis. Immunol Rev. 2020; 294(1):63-79. doi:10.1111/imr.12837
129. Zomer TP, Barendregt JNM, van Kooten B, van Bemmel T, Landman GW, van Hees BC, et al. Non-specific symptoms in adult patients referred to a Lyme centre. Clin Microbiol Infect. 2019; 25(1):67-70. doi: 10.1016/j.cmi.2018.09.016.
130. Miller JB, Aucott JN. Stages of Lyme Arthritis. J Clin Rheumatol. 2020. doi: 10.1097/RHU.0000000000001513.
131. Forrester JD, Meiman J, Mullins J, Nelson R, Ertel SH, Cartter M, et al. Notes from the field: update on Lyme carditis, groups at high risk, and frequency of associated sudden cardiac death--United States. MMWR Morb Mortal Wkly Rep. 2014; 63(43):982-3. PMID: 25356607.
132. Woolf PK, Lorsung EM, Edwards KS. Electrocardiographic findings in children with Lyme disease. Pediatr Emerg Care. 1991; 7:334–336. doi: 10.1097/00006565-199112000-00003.

133. Scheffold N, Herkommer B, Kandolf R, May AE. Lyme carditis--diagnosis, treatment and prognosis. Dtsch Arztebl Int. 2015; 112(12):202-8. doi: 10.3238/arztebl.2015.0202.
134. Clinckaert C, Bidgoli S, Verbeet T, Attou R, Gottignies P, Massaut J, et al. Peroperative cardiogenic shock suggesting acute coronary syndrome as initial manifestation of Lyme carditis. J Clin Anesth. 2016; 35:430-433. doi: 10.1016/j.jclinane.2016.08.005.
135. van der Linde MR. Lyme carditis: clinical characteristics of 105 cases. Scand J Infect Dis Suppl. 1991; 77:81-4. PMID: 1947815.
136. Kostić T, Momčilović S, Perišić ZD, Apostolović SR, Cvetković J, Jovanović A, et al. Manifestations of Lyme carditis. Int J Cardiol. 2017; 232:24-32. doi: 10.1016/j.ijcard.2016.12.169.
137. Bergler-Klein J, Ullrich R, Glogar D, Stanek G. Lyme-Borreliose und Kardiomyopathie [Lyme borreliosis and cardiomyopathy]. Wien Med Wochenschr. 1995; 145(7-8):196-8. PMID: 7610674.
138. Zagórski Z, Biziorek B, Haszcz D. Ophthalmic manifestations in Lyme borreliosis]. Przegl Epidemiol. 2002; 56 Suppl 1:85-90. PMID: 12194235.
139. Oteo JA, Martinez de Artola V, Maravi E, Eiros JM. Lyme disease and uveitis. Ann Intern Med. 1990; 112(11):883. doi: 10.7326/0003-4819-112-11-883.
140. Lambert JS. An overview of tick-borne infections in pregnancy and outcomes in the newborn: the need for prospective studies. Front Med (Lausanne). 2020; 7:72. doi:10.3389/fmed.2020.00072.
141. Waddell LA, Greig J, Lindsay LR, Hinckley AF, Ogden NH. A systematic review on the impact of gestational Lyme disease in humans on the fetus and newborn. PLoS One. 2018; 13(11):e0207067. doi: 10.1371/journal.pone.0207067.
142. Oliveira CR, Shapiro ED. Update on persistent symptoms associated with Lyme disease. Curr Opin Pediatr. 2015; 27(1):100-4. doi: 10.1097/MOP.0000000000000167.
143. Aguero-Rosenfeld ME, Nowakowski J, Bittker S, Cooper D, Nadelman RB, Wormser GP. Evolution of the serologic response to *Borrelia burgdorferi* in treated patients with culture-confirmed erythema migrans. J Clin Microbiol. 1996; 34(1):1-9. doi: 10.1128/jcm.34.1.1-9.1996.
144. Klempner MS, Hu LT, Evans J, Schmid CH, Johnson GM, Trevino RP, et al. Two controlled trials of antibiotic treatment in patients with persistent symptoms and a history of Lyme disease. N Engl J Med. 2001; 345(2):85-92. doi: 10.1056/NEJM200107123450202.
145. Krupp LB, Hyman LG, Grimson R, Coyle PK, Melville P, Ahnn S, et al. Study and treatment of post Lyme disease (STOP-LD): a randomized double masked clinical trial. Neurology. 2003; 60(12):1923-30. doi: 10.1212/01.wnl.0000071227.23769.9e.
146. Feder HM Jr, Johnson BJ, O'Connell S, Shapiro ED, Steere AC, Wormser GP, et al. A critical appraisal of "chronic Lyme disease". N Engl J Med. 2007; 357(14):1422-30. doi: 10.1056/NEJMra072023.
147. Shor S, Green C, Szantyr B, Phillips S, Liegner K, Burrascano JJ Jr, et al. Chronic Lyme disease: An evidence-based definition by the ILADS working group. Antibiotics (Basel). 2019; 8(4):269. doi: 10.3390/antibiotics8040269.
148. Barbour AG. Isolation and cultivation of Lyme disease spirochetes. Yale J Biol Med. 1984; 57(4):521-5. PMID: 6393604.
149. Pollack RJ, Telford SR 3rd, Spielman A. Standardization of medium for culturing Lyme disease spirochetes. J Clin Microbiol. 1993; 31(5):1251-5. doi: 10.1128/jcm.31.5.1251-1255.1993.
150. Ružić-Sabljić E, Maraspin V, Stupica D, Rojko T, Bogovič P, Strle F, et al. Comparison of MKP and BSK-H media for the cultivation and isolation of *Borrelia burgdorferi* sensu lato. PLoS One. 2017; 12(2):e0171622. doi: 10.1371/journal.pone.0171622.
151. Berger BW, Johnson RC, Kodner C, Coleman L. Cultivation of *Borrelia burgdorferi* from erythema migrans lesions and perilesional skin. J Clin Microbiol. 1992; 30(2):359-61. doi: 10.1128/jcm.30.2.359-361.1992.
152. Warkel RL, Luna LG, Helwig EB. A modified Warthin-Starry procedure at low pH for melanin. Am J Clin Pathol. 1980; 73(6):812-5. doi: 10.1093/ajcp/73.6.812.
153. Duray, P. H., A. Kusnitz, and R. Ryan. 1985. Demonstration of the Lyme disease spirochete Borrelia burgdorferi by a modification of the Dieterle stain. Lab. Med. 16:685-687.
154. De Koning J, Bosma RB, Hoogkamp-Korstanje JA. Demonstration of spirochaetes in patients with Lyme disease with a modified silver stain. J Med Microbiol. 1987; 23(3):261-7. doi: 10.1099/00222615-23-3-261.
155. Wilske B, Schriefer ME. Borrelia. In: Murray PR, Baron EJ, Jorgensen JH, Pfaller MA, Yolken RH, eds. Manual of clinical microbiology. Washington, DC: ASM Press, 2003; 937–954.
156. Persing DH, Rutledge BJ, Rys PN, Podzorski DS, Mitchell PD, Reed KD, Liu B, Fikrig E, Malawista SE. Target imbalance: disparity of Borrelia burgdorferi genetic material in synovial fluid from Lyme arthritis patients. J Infect Dis. 1994; 169(3):668-72. doi: 10.1093/infdis/169.3.668

157. CDC. Notice to Readers: Caution Regarding Testing for Lyme Disease. MMWR Morb Mortal Wkly Rep. 2005; 54:125.
158. Ružić-Sabljić E, Cerar T. Progress in the molecular diagnosis of Lyme disease. Expert Rev Mol Diagn. 2017; 17(1):19-30. doi: 10.1080/14737159.2016.1246959.
159. Aguero-Rosenfeld ME, Wang G, Schwartz I, Wormser GP. Diagnosis of lyme borreliosis. Clin Microbiol Rev. 2005; 18(3):484-509. doi: 10.1128/CMR.18.3.484-509.2005
160. Aguero-Rosenfeld ME, Nowakowski J, McKenna DF, Carbonaro CA, Wormser GP. Serodiagnosis in early Lyme disease. J Clin Microbiol. 1993; 31(12):3090-5. doi: 10.1128/jcm.31.12.3090-3095.1993. Erratum in: J Clin Microbiol 1994 Mar;32(3):860.
161. Aucott J, Morrison C, Munoz B, Rowe PC, Schwarzwalder A, West SK. Diagnostic challenges of early Lyme disease: lessons from a community case series. BMC Infect Dis. 2009; 9:79. doi: 10.1186/1471-2334-9-79.
162. Fikrig E, Barthold SW, Sun W, Feng W, Telford SR 3rd, Flavell RA. Borrelia burgdorferi P35 and P37 proteins, expressed in vivo, elicit protective immunity. Immunity. 1997; 6(5):531-9. doi: 10.1016/s1074-7613(00)80341-6. Erratum in: Immunity 1998 Nov;9(5):755. PMID: 9175831.
163. Strle F, Stanek G. Clinical manifestations and diagnosis of lyme borreliosis. Curr Probl Dermatol. 2009; 37:51-110. doi: 10.1159/000213070.
164. Kalish RA, McHugh G, Granquist J, Shea B, Ruthazer R, Steere AC. Persistence of immunoglobulin M or immunoglobulin G antibody responses to *Borrelia burgdorferi* 10-20 years after active Lyme disease. Clin Infect Dis. 2001; 33(6):780-5. doi: 10.1086/322669.
165. Hernández-Novoa B, Orduña A, Bratos MA, Eiros JM, Fernández JM, Gutiérrez MP, et al. Utility of a commercial immunoblot kit (BAG-Borrelia blot) in the diagnosis of the preliminary stages of Lyme disease. Diagn Microbiol Infect Dis. 2003; 47(1):321-9. doi: 10.1016/s0732-8893(03)00111-1.
166. Enders G, Biber M, Baier R, Hlobil H, Wellensiek HJ. Suspected syphilis during pregnancy due to cross reactions in *Borrelia* infection. Dtsch Med Wochenschr. 1988;113(39):1511-4. doi: 10.1055/s-2008-1067843.
167. Strizova Z, Smrz D, Bartunkova J. Seroprevalence of *Borrelia* IgM and IgG antibodies in healthy individuals: a caution against serology misinterpretations and unnecessary antibiotic treatments. Vector Borne Zoonotic Dis. 2020; 20(10):800-802. doi: 10.1089/vbz.2020.2632.
168. Panelius J, Lahdenne P, Saxén H, Carlsson SA, Heikkilä T, Peltomaa M, et al. Diagnosis of Lyme neuroborreliosis with antibodies to recombinant proteins DbpA, BBK32, and OspC, and VlsE IR6 peptide. J Neurol. 2003; 250(11):1318-27. doi: 10.1007/s00415-003-0205-2.
169. Raoult D, Hechemy KE, Baranton G. Cross-reaction with Borrelia burgdorferi antigen of sera from patients with human immunodeficiency virus infection, syphilis, and leptospirosis. J Clin Microbiol. 1989; 27(10):2152-5. doi: 10.1128/jcm.27.10.2152-2155.1989.
170. Magnarelli LA, Miller JN, Anderson JF, Riviere GR. Cross-reactivity of nonspecific treponemal antibody in serologic tests for Lyme disease. J Clin Microbiol. 1990; 28(6):1276-9. doi: 10.1128/jcm.28.6.1276-1279.1990.
171. Tuuminen T, Hedman K, Söderlund-Venermo M, Seppälä I. Acute parvovirus B19 infection causes nonspecificity frequently in Borrelia and less often in Salmonella and Campylobacter serology, posing a problem in diagnosis of infectious arthropathy. Clin Vaccine Immunol. 2011 Jan;18(1):167-72. doi: 10.1128/CVI.00367-10.
172. Liang FT, Aberer E, Cinco M, Gern L, Hu CM, Lobet YN, et al. Antigenic conservation of an immunodominant invariable region of the VlsE lipoprotein among European pathogenic genospecies of *Borrelia burgdorferi* SL. J Infect Dis. 2000; 182(5):1455-62. doi: 10.1086/315862
173. Goettner G, Schulte-Spechtel U, Hillermann R, Liegl G, Wilske B, Fingerle V. Improvement of Lyme borreliosis serodiagnosis by a newly developed recombinant immunoglobulin G (IgG) and IgM line immunoblot assay and addition of VlsE and DbpA homologues. J Clin Microbiol. 2005; 43(8):3602-9. doi: 10.1128/JCM.43.8.3602-3609.2005.
174. Krause PJ, Narasimhan S, Wormser GP, Barbour AG, Platonov AE, Brancato J, et al. *Borrelia miyamotoi* sensu lato seroreactivity and seroprevalence in the northeastern United States. Emerg Infect Dis. 2014; 20(7):1183-90. doi: 10.3201/eid2007.131587.
175. Koetsveld J, Platonov AE, Kuleshov K, Wagemakers A, Hoornstra D, Ang W, et al. Borrelia miyamotoi infection leads to cross-reactive antibodies to the C6 peptide in mice and men. Clin Microbiol Infect. 2020; 26(4):513.e1-513.e6. doi: 10.1016/j.cmi.2019.07.026.
176. Leeflang MM, Ang CW, Berkhout J, Bijlmer HA, Van Bortel W, Brandenburg AH, et al. The diagnostic accuracy of serological tests for Lyme borreliosis in Europe: a systematic review and meta-analysis. BMC Infect Dis. 2016; 16:140. doi: 10.1186/s12879-016-1468-4.
177. Hunfeld KP, Stanek G, Straube E, Hagedorn HJ, Schörner C, Mühlschlegel F, et al. Quality of Lyme

disease serology. Lessons from the German Proficiency Testing Program 1999-2001. A preliminary report. Wien Klin Wochenschr. 2002; 114(13-14):591-600. PMID: 12422607.

178. Lohr B, Fingerle V, Norris DE, Hunfeld KP. Laboratory diagnosis of Lyme borreliosis: Current state of the art and future perspectives. Crit Rev Clin Lab Sci. 2018 Jun;55(4):219-245. doi: 10.1080/10408363.2018.1450353.
179. Tilton R. Laboratory aids for the diagnosis of *Borrelia burgdorferi* infection. J Spiroch Tick-Borne Dis 1994; 1:18-23.
180. Dressler F, Whalen JA, Reinhardt BN, Steere AC. Western blotting in the serodiagnosis of Lyme disease. J Infect Dis. 1993; 167(2):392-400. doi: 10.1093/infdis/167.2.392.
181. Engstrom SM, Shoop E, Johnson RC. Immunoblot interpretation criteria for serodiagnosis of early Lyme disease. J Clin Microbiol. 1995 Feb;33(2):419-27. doi: 10.1128/jcm.33.2.419-427.1995.
182. Branda JA, Strle F, Strle K, Sikand N, Ferraro MJ, Steere AC. Performance of United States serologic assays in the diagnosis of Lyme borreliosis acquired in Europe. Clin Infect Dis. 2013; 57(3):333-40. doi: 10.1093/cid/cit235.
183. Reiber H, Peter JB. Cerebrospinal fluid analysis: disease-related data patterns and evaluation programs. J Neurol Sci. 2001; 184(2):101-22. doi: 10.1016/s0022-510x(00)00501-3.
184. Blanc F, Jaulhac B, Fleury M, de Seze J, de Martino SJ, Remy V, et al. Relevance of the antibody index to diagnose Lyme neuroborreliosis among seropositive patients. Neurology. 2007; 69(10):953-8. doi: 10.1212/01.wnl.0000269672.17807.e0.
185. Focosi D, Bestagno M, Burrone O, Petrini M. CD57+ T lymphocytes and functional immune deficiency. J Leukoc Biol. 2010; 87(1):107-16. doi: 10.1189/jlb.0809566.
186. Stricker RB, Winger EE. Decreased CD57 lymphocyte subset in patients with chronic Lyme disease. Immunol Lett. 2001; 76(1):43-8. doi: 10.1016/s0165-2478(00)00316-3.
187. Marques A, Brown MR, Fleisher TA. Natural killer cell counts are not different between patients with post-Lyme disease syndrome and controls. Clin Vaccine Immunol. 2009; 16(8):1249-50. doi: 10.1128/CVI.00167-09.
188. Kared H, Martelli S, Ng TP, Pender SL, Larbi A. CD57 in human natural killer cells and T-lymphocytes. Cancer Immunol Immunother. 2016; 65(4):441-52. doi: 10.1007/s00262-016-1803-z.
189. Nielsen CM, White MJ, Goodier MR, Riley EM. Functional Significance of CD57 Expression on Human NK Cells and Relevance to Disease. Front Immunol. 2013; 4:422. doi: 10.3389/fimmu.2013.00422.
190. Nordberg M, Forsberg P, Nyman D, Skogman BH, Nyberg C, Ernerudh J, et al. Can ELISPOT be applied to a clinical setting as a diagnostic utility for neuroborreliosis? Cells. 2012; 1(2):153-67. doi: 10.3390/cells1020153.
191. Callister SM, Jobe DA, Stuparic-Stancic A, Miyamasu M, Boyle J, Dattwyler RJ, et al. Detection of IFN-γ secretion by T cells collected before and after successful treatment of early Lyme disease. Clin Infect Dis. 2016; 62(10):1235-1241. doi: 10.1093/cid/ciw112.
192. van Gorkom T, Sankatsing SUC, Voet W, Ismail DM, Muilwijk RH, Salomons M, et al. An enzyme-linked immunosorbent spot assay measuring *Borrelia burgdorferi* B31-specific interferon gamma-secreting T cells cannot discriminate active Lyme neuroborreliosis from past Lyme borreliosis: a prospective study in the Netherlands. J Clin Microbiol. 2018; 56(4):e01695-17. doi: 10.1128/JCM.01695-17.
193. Jacek E, Fallon BA, Chandra A, Crow MK, Wormser GP, Alaedini A. Increased IFNα activity and differential antibody response in patients with a history of Lyme disease and persistent cognitive deficits. J Neuroimmunol. 2013; 255(1-2):85-91. doi: 10.1016/j.jneuroim.2012.10.011.
194. Koedel U, Fingerle V, Pfister HW. Lyme neuroborreliosis-epidemiology, diagnosis and management. Nat Rev Neurol. 2015; 11(8):446-56. doi: 10.1038/nrneurol.2015.121
195. Yang J, Han X, Liu A, Bao F, Peng Y, Tao L, et al. Chemokine CXC ligand 13 in cerebrospinal fluid can be used as an early diagnostic biomarker for Lyme neuroborreliosis: a meta-analysis. J Interferon Cytokine Res. 2017; 37(10):433-439. doi: 10.1089/jir.2016.0101.
196. Rupprecht TA, Manz KM, Fingerle V, Lechner C, Klein M, Pfirrmann M, Koedel U. Diagnostic value of cerebrospinal fluid CXCL13 for acute Lyme neuroborreliosis. A systematic review and meta-analysis. Clin Microbiol Infect. 2018; 24(12):1234-1240. doi: 10.1016/j.cmi.2018.04.007.
197. Aucott JN, Soloski MJ, Rebman AW, Crowder LA, Lahey LJ, Wagner CA, et al. CCL19 as a chemokine risk factor for posttreatment Lyme disease syndrome: a prospective clinical cohort study. Clin Vaccine Immunol. 2016; 23(9):757-66. doi: 10.1128/CVI.00071-16.
198. Crowley JT, Drouin EE, Pianta A, Strle K, Wang Q, Costello CE, et al. A highly expressed human protein, apolipoprotein B-100, serves as an autoantigen in asSubgroup of patients with Lyme disease. J Infect Dis. 2015 Dec 1;212(11):1841-50. doi: 10.1093/infdis/jiv310.

199. Strle K, Sulka KB, Pianta A, Crowley JT, Arvikar SL, Anselmo A, et al. T-Helper 17 cell cytokine responses in Lyme disease correlate with *Borrelia burgdorferi* antibodies during early infection and with autoantibodies late in the illness in patients with antibiotic-refractory Lyme arthritis. Clin Infect Dis. 2017; 64(7):930-938. doi: 10.1093/cid/cix002.
200. Steere AC. Lyme disease. N Engl J Med. 1989; 321(9):586-96. doi: 10.1056/NEJM198908313210906.
201. Oteo JA, Martínez de Artola V. Tratamiento de la infección por *Borrelia burgdorferi* [Treatment of *Borrelia burgdorferi* infection]. Enferm Infecc Microbiol Clin. 1991; 9(1):52-4. PMID: 2029560.
202. Torbahn G, Hofmann H, Rücker G, Bischoff K, Freitag MH, Dersch R, et al. Efficacy and safety of antibiotic therapy in early cutaneous Lyme borreliosis: A network meta-analysis. JAMA Dermatol. 2018; 154(11):1292-1303. doi: 10.1001/jamadermatol.2018.3186.
203. Stricker RB. Counterpoint: long-term antibiotic therapy improves persistent symptoms associated with lyme disease. Clin Infect Dis. 2007; 45(2):149-57. doi: 10.1086/518853.
204. Lantos PM. Chronic Lyme disease. Infect Dis Clin North Am. 2015; 29(2):325-40. doi: 10.1016/j.idc.2015.02.006.
205. Maraspin V, Cimperman J, Lotric-Furlan S, Logar M, Ruzić-Sabljić E, Strle F. Erythema migrans in solid-organ transplant recipients. Clin Infect Dis. 2006; 42(12):1751-4. doi: 10.1086/504384.
206. Boršič K, Blagus R, Cerar T, Strle F, Stupica D. Clinical course, serologic response, and long-term outcome in elderly patients with early Lyme borreliosis. J Clin Med. 2018; 7(12):506. doi: 10.3390/jcm7120506.
207. Maraspin V, Bogovič P, Rojko T, Ogrinc K, Ružić-Sabljić E, Strle F. Early Lyme borreliosis in patients treated with Tumour Necrosis Factor-Alfa Inhibitors. J Clin Med. 2019; 8(11):1857. doi: 10.3390/jcm8111857.
208. Maraspin V, Bogovič P, Rojko T, Ružić-Sabljić E, Strle F. Erythema Migrans: Course and outcome in patients treated with rituximab. Open Forum Infect Dis. 2019; 6(7):ofz292. doi: 10.1093/ofid/ofz292.
209. Stupica D, Velušcek M, Blagus R, Bogovic P, Rojko T, Cerar T, et al. Oral doxycycline versus intravenous ceftriaxone for treatment of multiple erythema migrans: an open-label alternate-treatment observational trial. J Antimicrob Chemother. 2018; 73(5):1352-1358. doi: 10.1093/jac/dkx534.
210. Kortela E, Kanerva MJ, Puustinen J, Hurme S, Airas L, Lauhio A, et al. Oral doxycycline compared to intravenous ceftriaxone in the treatment of Lyme neuroborreliosis: A multicenter, equivalence, randomized, open-label trial. Clin Infect Dis. 2021; 72(8):1323-1331. doi: 10.1093/cid/ciaa217.
211. Muanda FT, Sheehy O, Bérard A. Use of antibiotics during pregnancy and risk of spontaneous abortion. CMAJ. 2017; 189(17):E625-E633. doi: 10.1503/cmaj.161020.
212. Muanda FT, Sheehy O, Bérard A. Use of antibiotics during pregnancy and the risk of major congenital malformations: a population based cohort study. Br J Clin Pharmacol. 2017; 83(11):2557-2571. doi: 10.1111/bcp.13364.
213. Meissner HC. When can doxycycline be used in young children? https://www.aappublications.org/news/2020/02/27/idsnapshot022720
214. Dattwyler RJ, Volkman DJ, Conaty SM, Platkin SP, Luft BJ. Amoxycillin plus probenecid versus doxycycline for treatment of erythema migrans borreliosis. Lancet. 1990; 336(8728):1404-6. doi: 10.1016/0140-6736(90)93103-v.
215. Massarotti EM, Luger SW, Rahn DW, Messner RP, Wong JB, Johnson RC, et al. Treatment of early Lyme disease. Am J Med. 1992; 92(4):396-403. doi: 10.1016/0002-9343(92)90270-l
216. Nadelman RB, Luger SW, Frank E, Wisniewski M, Collins JJ, Wormser GP. Comparison of cefuroxime axetil and doxycycline in the treatment of early Lyme disease. Ann Intern Med. 1992; 117(4):273-80. doi: 10.7326/0003-4819-117-4-273.
217. Luger SW, Paparone P, Wormser GP, Nadelman RB, Grunwaldt E, Gomez G, et al. Comparison of cefuroxime axetil and doxycycline in treatment of patients with early
Lyme disease associated with erythema migrans. Antimicrob Agents Chemother. 1995; 39(3):661-7. doi: 10.1128/AAC.39.3.661.
218. Luft BJ, Dattwyler RJ, Johnson RC, Luger SW, Bosler EM, Rahn DW, et al. Azithromycin compared with amoxicillin in the treatment of erythema migrans. A double-blind, randomized, controlled trial. Ann Intern Med. 1996; 124(9):785-91. doi: 10.7326/0003-4819-124-9-199605010-00002.
219. Maraspin V, Lusa L, Blejec T, Ružić-Sabljić E, Pohar Perme M, Strle F. Course and outcome of Erythema Migrans in pregnant women. J Clin Med 2020; 24; 9:2364. doi: 10.3390/jcm9082364.

220. Maraspin V, Nahtigal Klevišar M, Ružić-Sabljić E, Lusa L, Strle F. Borrelial Lymphocytoma in Adult Patients. Clin Infect Dis. 2016; 63(7):914-21. doi: 10.1093/cid/ciw417.
221. Dattwyler RJ, Luft BJ, Kunkel MJ, Finkel MF, Wormser GP, Rush TJ, et al. Ceftriaxone compared with doxycycline for the treatment of acute disseminated Lyme disease. N Engl J Med. 1997; 337(5):289-94. doi: 10.1056/NEJM199707313370501
222. Kowalski TJ, Tata S, Berth W, Mathiason MA, Agger WA. Antibiotic treatment duration and long-term outcomes of patients with early lyme disease from a lyme disease-hyperendemic area. Clin Infect Dis. 2010; 50(4):512-20. doi: 10.1086/649920.
223. Avellan S, Bremell D. Adjunctive Corticosteroids for Lyme Neuroborreliosis Peripheral Facial Palsy - a prospective study with historical controls. Clin Infect Dis. 2021:ciab370. doi: 10.1093/cid/ciab370.
224. Halperin JJ, Shapiro ED, Logigian E, Belman AL, Dotevall L, Wormser GP, et al. Practice parameter: treatment of nervous system Lyme disease (an evidence-based review): report of the Quality Standards Subcommittee of the American Academy of Neurology. Neurology. 2007; 69(1):91-102. doi: 10.1212/01.wnl.0000265517.66976.28. Erratum in: Neurology. 2008; 70(14):1223. PMID: 17522387.
225. Steere AC, Hutchinson GJ, Rahn DW, Sigal LH, Craft JE, DeSanna ET, et al. Treatment of the early manifestations of Lyme disease. Ann Intern Med. 1983; 99(1):22-6. doi: 10.7326/0003-4819-99-1-22.
226. Kaplan RF, Trevino RP, Johnson GM, Levy L, Dornbush R, Hu LT, et al. Cognitive function in post-treatment Lyme disease: do additional antibiotics help? Neurology. 2003; 60(12):1916-22. doi: 10.1212/01.wnl.0000068030.26992.25.
227. Fallon BA, Keilp JG, Corbera KM, Petkova E, Britton CB, Dwyer E, et al. A randomized, placebo-controlled trial of repeated IV antibiotic therapy for Lyme encephalopathy. Neurology. 2008; 70(13):992-1003. doi: 10.1212/01.WNL.0000284604.61160.2d.
228. Halperin JJ. Prolonged Lyme disease treatment: enough is enough. Neurology. 2008; 70(13):986-7. doi: 10.1212/01.WNL.0000291407.40667.69.
229. Berende A, ter Hofstede HJ, Vos FJ, van Middendorp H, Vogelaar ML, Tromp M, et al. Randomized trial of longer-term therapy for symptoms attributed to Lyme disease. N Engl J Med. 2016; 374(13):1209-20. doi: 10.1056/NEJMoa1505425.
230. García-Alvarez L, Oteo JA. Efectos no antimicrobianos de las tetraciclinas [Nonantimicrobial effects of tetracyclines]. Rev Esp Quimioter. 2010; 23(1):4-11. PMID: 20232018.
231. García-Alvarez L, Palomar AM, Oteo JA. Prevention and prophylaxis of tick bites and tick-borne related diseases. Am J Infect Dis. 2013; 9(3), 104-116. https://doi.org/10.3844/ajidsp.2013.104.116.
232. Vaughn MF, Meshnick SR. Pilot study assessing the effectiveness of long-lasting permethrin-impregnated clothing for the prevention of tick bites. Vector Borne Zoonotic Dis. 2011; 11(7):869-75. doi: 10.1089/vbz.2010.0158.
233. Faulde MK, Rutenfranz M, Keth A, Hepke J, Rogge M, Görner A. Pilot study assessing the effectiveness of factory-treated, long-lasting permethrin-impregnated clothing for the prevention of tick bites during occupational tick exposure in highly infested military training areas, Germany. Parasitol Res. 2015; 114(2):671-8. doi: 10.1007/s00436-014-4232-y.
234. Miller NJ, Rainone EE, Dyer MC, González ML, Mather TN. Tick-bite protection with permethrin-treated summer-weight clothing. J Med Entomol. 2011; 48(2):327-33. doi: 10.1603/me10158.
235. Büchel K, Bendin J, Gharbi A, Rahlenbeck S, Dautel H. Repellent efficacy of DEET, Icaridin, and EBAAP against *Ixodes ricinus* and *Ixodes scapularis* nymphs (Acari, Ixodidae). Ticks Tick Borne Dis. 2015; 6(4):494-8. doi: 10.1016/j.ttbdis.2015.03.019.
236. Katz TM, Miller JH, Hebert AA. Insect repellents: historical perspectives and new developments. J Am Acad Dermatol. 2008; 58(5):865-71. doi: 10.1016/j.jaad.2007.10.005.
237. https://enfamilia.aeped.es/prevencion/repelentes-insectos
238. https://wwwnc.cdc.gov/travel/yellowbook/2020/noninfectious-health-risks/mosquitoes-ticks-and-other-arthropods
239. Shaw SE, Day MJ, Birtles RJ, Breitschwerdt EB. Tick-borne infectious diseases of dogs. Trends Parasitol. 2001; 17(2):74-80. doi: 10.1016/s1471-4922(00)01856-0.
240. Berrada ZL, Telford SR. Burden of tick-borne infections on American companion animals. Top Companion Anim Med. 2009; 24(4):175-81. doi: 10.1053/j.tcam.2009.06.005.

241. Poland GA. Vaccines against Lyme disease: What happened and what lessons can we learn? Clin Infect Dis. 2011; 52 Suppl 3:s253-8. doi: 10.1093/cid/ciq116.
242. Shen AK, Mead PS, Beard CB. The Lyme disease vaccine--a public health perspective. Clin Infect Dis. 2011; 52 Suppl 3:s247-52. doi: 10.1093/cid/ciq115.
243. Richardson M, Khouja C, Sutcliffe K. Interventions to prevent Lyme disease in humans: A systematic review. Prev Med Rep. 2018; 13:16-22. doi: 10.1016/j.pmedr.2018.11.004.
244. Hofhuis A, Herremans T, Notermans DW, Sprong H, Fonville M, van der Giessen JW, et al. A prospective study among patients presenting at the general practitioner with a tick bite or erythema migrans in The Netherlands. PLoS One. 2013; 8(5):e64361. doi: 10.1371/journal.pone.0064361.
245. Wilhelmsson P, Fryland L, Lindblom P, Sjöwall J, Ahlm C, Berglund J, et al. A prospective study on the incidence of *Borrelia burgdorferi* sensu lato infection after a tick bite in Sweden and on the Åland Islands, Finland (2008-2009). Ticks Tick Borne Dis. 2016; 7(1):71-79. doi: 10.1016/j.ttbdis.2015.08.009.
246. Oteo JA, Martínez de Artola V, Gómez-Cadiñanos R, Casas JM, Blanco JR, Rosel L. Evaluación de los métodos de retirada de garrapatas en la ixodidiasis humana [Evaluation of methods of tick removal in human ixodidiasis]. Rev Clin Esp. 1996; 196(9):584-7. PMID: 8966318.
247. Oteo JA, Blanco JR, Ibarra V. ¿Podemos prevenir las enfermedades transmitidas por garrapatas? [Can we prevent tick-borne transmitted diseases?]. Enferm Infecc Microbiol Clin. 2001; 19(10):509-13. doi: 10.1016/s0213-005x(01)72718-8.
248. Needham GR. Evaluation of five popular methods for tick removal. Pediatrics. 1985; 75(6):997-1002. PMID: 4000801.
249. Piesman J, Dolan MC. Protection against lyme disease spirochete transmission provided by prompt removal of nymphal *Ixodes scapularis* (Acari: Ixodidae). J Med Entomol. 2002; 39(3):509-12. doi: 10.1603/0022-2585-39.3.509.
250. Zenner L, Drevon-Gaillot E, Callait-Cardinal MP. Evaluation of four manual tick-removal devices for dogs and cats. Vet Rec. 2006; 159(16):526-9. doi: 10.1136/vr.159.16.526.
251. Duscher GG, Peschke R, Tichy A. Mechanical tools for the removal of Ixodes ricinus female ticks--differences of instruments and pulling or twisting? Parasitol Res. 2012; 111(4):1505-11. doi: 10.1007/s00436-012-2987-6.
252. Oteo JA, Maraví E, Martínez de Artola V, Antuñano P. Parálisis por mordedura de garrapata [Paralysis caused by tick bite]. Med Clin (Barc). 1990; 94(7):275-6. PMID: 2325490.
253. Parola P, Raoult D. Ticks and tickborne bacterial diseases in humans: an emerging infectious threat. Clin Infect Dis. 2001; 32(6):897-928. doi: 10.1086/319347. Erratum in: Clin Infect Dis 2001; 33(5):749. PMID: 11247714
254. Wormser GP, Warshafsky S, Visintainer P. Aggregation of data from 4 clinical studies demonstrating efficacy of single-dose doxycycline postexposure for prevention of the spirochetal infections: Lyme disease, syphilis, and tick-borne relapsing fever. Diagn Microbiol Infect Dis. 2021; 99(4):115293. doi: 10.1016/j.diagmicrobio.2020.115293.
255. Wilske B. Epidemiology and diagnosis of Lyme borreliosis. Ann Med. 2005; 37(8):568-79. doi: 10.1080/07853890500431934.
256. Arms MG, Hofhuis A, Sprong H, Bennema SC, Ferreira JA, Fonville M, et al. A single dose of doxycycline after an *Ixodes ricinus* tick bite to prevent Lyme borreliosis: An open-label randomized controlled trial. J Infect. 2021; 82(1):98-104. doi: 10.1016/j.jinf.2020.06.032.