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Tick-borne diseases in Belgium:

The incidence and economic burden of Lyme borreliosis and the occurrence of other tick-borne infections

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It always seems impossible until it’s done

– Nelson Mandela -

Jury

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Table of contents

List of Abbreviations

ACA	Acrodermatitis Chronica Atrophicans
B. burgdorferi s.l.	*Borrelia burgdorferi* sensu lato
B. burgdorferi s.s.	*Borrelia burgdorferi* sensu stricto
BCPI	Belgian Centre for Pharmacotherapeutic Information
CFQ	Cognitive Failure Questionnaire
CI	Confidence Interval
CSF	Cerebrospinal Fluid
DALY	Disability-Adjusted Life Year
DISS	Disseminated/late Lyme borreliosis
DW	Disability Weight
ECDC	European Center for Disease Prevention and Control
EIA	Enzyme-ImmunoAssay
ELISA	Enzyme-Linked Immunosorbent Assay
EM	Erythema Migrans
GALI	Global Activity Limitation Index
GP	General Practitioner
ICD-10-CM	International Statistical Classification of Diseases - 10th revision – Clinical Modification
IDSA	Infectious Diseases Society of America
KCE	Belgian Health Care Knowledge Centre
LA	Lyme Arthritis
LB	Lyme Borreliosis
LNB	Lyme Neuroborreliosis
LTFU	Loss To Follow-Up
MCAR	Missing Completely At Random

MEM	Multiple Erythema Migrans
MFG / MFD	Minimal Financial Data
MZG / MCD	Minimal Clinical Data
NNT	Number-Needed-to-Treat
NRC	National Reference Centers
OR	Odds Ratio
PCR	Polymerase Chain Reaction
PLDS	Post-Lyme Disease Syndrome
PTLDS	Post-Treatment Lyme Disease Syndrome
RIVM	Laboratory for Zoonosis and Environmental Microbiology
RIZIV	National Institute for Health and Disability Insurance
RR	Risk Ratio
SF-36 BP	SF-36 Bodily Pain
SF-36 VT	SF-36 Vitality
SGP	Sentinel General Practices
T0	Time of diagnosis
T3	3 months after diagnosis
T6, T12, T24	6, 12, 24 months after treatment
TBE	Tick-Borne Encephalitis
TBEV	Tick-Borne Encephalitis Virus
UI	Uncertainty Interval
YLD	Years Lived with Disability
YLL	Years of Life Lost due to mortality

Chapter 1

Introduction

1.1. Lyme borreliosis

Lyme borreliosis (LB) is a tick-borne zoonotic disease first described in 1976 after an outbreak of oligoarthritis cases in Lyme, Connecticut, United States [1]. The causative agent, a group of spirochete bacteria later named *Borrelia burgdorferi* sensu lato (s.l.), was isolated from ticks and humans in the early 1980s [2–4]. Following this discovery, several neurological and dermatological manifestations earlier described in Europe could likewise be linked to this new pathogen, with a first report of erythema migrans (EM), the red expanding rash, dating back to 1910 [5]. Since then, at least 20 different genospecies of the *B. burgdorferi* s.l. complex have been identified, yet not all are pathogenic. In Europe, at least five are: *B. afzelii* and *B. garinii*, which are most prevalent, and *B. burgdorferi* sensu stricto (s.s.), *B. bavariensis* and *B. spielmanii* [6–8]. Other pathogens (*B. bissetii*, *B. valaisiana* and *B. lusitaniae*) have only been identified in single cases. In the United States, LB is predominantly caused by *B. burgdorferi* s.s. [6–8]. At date, LB is the most prevalent tick-borne disease in the Northern Hemisphere.

1.1.1 Ticks and pathogen transmission

B. burgdorferi s.l. is transmitted to humans by hard ticks, predominantly by *Ixodes ricinus* in Europe and *I. scapularis* and *I. pacificus* in North America. The life cycle of these ticks consists of a passive egg stage and three active stages: larva, nymph and adult. One blood meal is needed during each active stage in order to develop into the next stage or to lay eggs (adult females); adult male ticks only sporadically feed [7]. A tick can acquire *Borrelia* spirochetes while feeding on an infected reservoir host, and after molting into a new tick stage, they can transmit the spirochetes to a new host during a subsequent blood meal [8,9]. Larvae and nymphs often feed on reservoir hosts such as rodents or birds. Adult ticks feed on larger animals such as deer, which are not competent for *B. burgdorferi* s.l. transmission but play an important role in the maintenance of the tick population [7,9]. Humans are an accidental host that can be bitten by each tick stage. The full development process of *I. ricinus* usually takes two to

three years but can take up to six years, depending on, amongst others, the climate and host availability [10].

Humans can get infected with *B. Burgdorferi* s.l. bacteria through the bite of an infected tick. When feeding, the spirochetes migrate from the tick's midgut to the salivary glands [8]. The risk of transmitting the bacteria increases with increasing tick attachment time to the skin [8,11]. A Belgian study on ticks collected from humans in 2017, found 14% of ticks to be infected with *B. burgdorferi* s.l. [12]. Adult ticks were more often infected (20%) than nymphs (12%), which can be explained by the additional blood meal they have taken [12]. As vertical transmission, i.e., transmission from female adult to eggs, of *B. Burgdorferi* s.l. is rare or non-existent, larvae are rarely infected [7].

Transmission of *B. burgdorferi* s.l. from mother to fetus has only been reported in a few single cases, yet reliable studies on the potential impact of gestational LD on the fetus are limited [8,13]. Epidemiological studies comparing exposed vs. unexposed populations did not show an association between LB in the mother and adverse birth outcomes [13–19]. Yet, treatment of LB in pregnant women remains important [13,15].

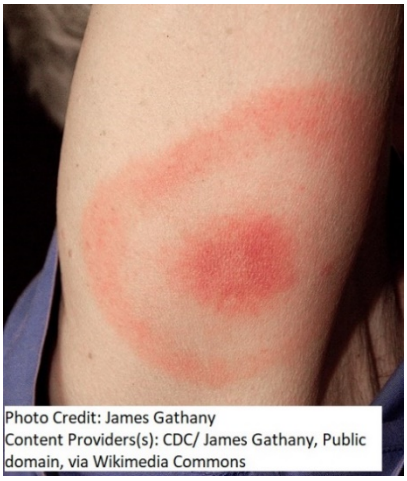
1.1.2 Clinical manifestations

Even when *B. burgdorferi* s.l. is transmitted to a person, it does not necessarily cause LB. Asymptomatic infections have been reported to be relatively common in Europe, possibly as common as clinical LB [20–24]. In general, the risk of LB after a tick bite has been estimated to be low, between 1 and 5% [20,23–26]. When LB develops, the most common clinical manifestation is an erythema migrans, an early localized infection occurring within days to weeks at the site of the tick bite (Figure 1A). The lesion starts as a red macule or papule and expands over days to weeks into a red or bluish-red patch, usually round or oval; a typical central clearing may be present but homogeneous lesions also occur [27,28]. The lesion can also be atypical (e.g., with vesicles or papules). An EM may or may not be accompanied by mild local symptoms

(itching, burning or pain) or by systemic symptoms such as fatigue, myalgia, arthralgia, headache or fever [8,29,30]. Even without treatment, EM disappears spontaneously [8]. EM should be distinguished from a local hypersensitivity reaction to the bite for which redness occurs sooner (< 2 days) and is not progressively expanding [31].

Figure 1. Dermatological manifestations of LB

A.



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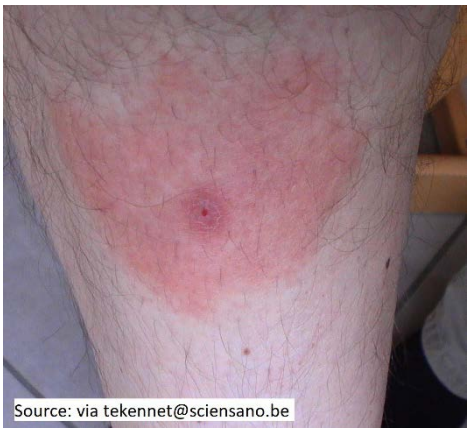

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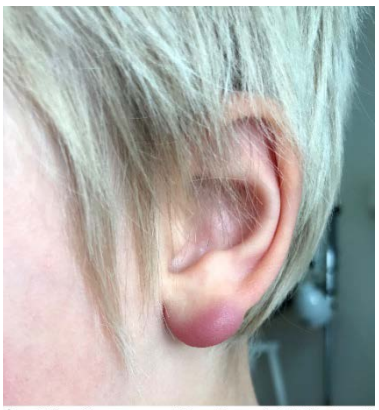
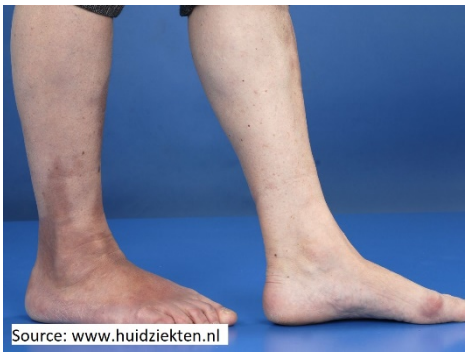
B.



Gzzz(https://commons.wikimedia.org/wiki/File:Borrelial_lymphocytoma_2.jpg), CC BY-SA 4.0 <https://creativecommons.org/licenses/by-sa/4.0>, via Wikimedia Commons

C.



Source: www.huidziekten.nl

A. Erythema migrans, with and without central clearing; B. Borrelial lymphocytoma;
C. Acrodermatitis chronica atrophicans

Another early, yet rare, skin manifestation of LB is borrelial lymphocytoma which is a blue-red swelling/nodule of 1-5 cm occurring 1-6 months after the bite, usually on an ear lobe, nipple or scrotum (Figure 1B) [8,29,30].

If early localized LB is left untreated, but also without preceding EM, *Borrelia* spirochetes can spread throughout the body and cause early disseminated LB, weeks to months after the bite, or late LB, months to years after the bite [8]. This way LB can further affect the skin with multiple erythema migrans (MEM) as an early disseminated manifestation or acrodermatitis chronica atrophicans (ACA) as a late manifestation. ACA is a bluish-red skin lesion with swelling, mostly at distal parts of the limbs, which later becomes atrophic (Figure 1C) [8,32]. ACA can develop up to eight years after the initial infection and almost exclusively occurs in Europe; it can be accompanied with peripheral neuropathy and joint pain [28,32,33]. When *B. burgdorferi* s.l. spreads to the nervous system, early Lyme neuroborreliosis (LNB) can develop. In adults in Europe, it mainly manifests with painful meningoradiculitis (Bannwarth syndrome) which involves radicular pain that is usually severe and worse at night and/or paresis. The latter possibly affects cranial nerves (frequently facial nerve), the abdominal wall or lower limbs [8,34]. In combination with these symptoms, headache is often present. Central nervous system manifestations are rare. In children, acute facial palsy is most common [34]. Late LNB, with symptoms during more than six months, is rare [8,34]. Persistent or recurrent joint swelling of one or a few joints, commonly the knee, are the most common symptoms of Lyme arthritis, a late manifestation of LB (months to years after the infection), which without treatment may persist for years [29]. More rarely, LB can affect the heart, referred to as Lyme carditis, with acute onset of atrioventricular (I-III) block as the principal manifestation [8,29]. Ocular manifestations are also rare and usually present with conjunctivitis [29].

Although all pathogenic *B. burgdorferi* s.l. genospecies can cause erythema migrans, some disease manifestations are found to be more often associated with specific genospecies which explains existing differences in the occurrence of clinical

manifestations in different geographical areas [8,35]. In North America, LA is more common as it is more often associated with *B. burgdorferi* s.s., whereas genospecies in Europe are more often associated with skin manifestations (*B. afzelii*) and LNB (*B. garinii* & B. *bavariensis*) [36–39].

1.1.3 Diagnosis and treatment

Diagnosis of LB is purely clinical for erythema migrans as in this early stage antibodies have not yet developed and sensitivity of serological tests is low (around 50%) [28,29,40]. In contrast, the diagnosis of disseminated or late LB is based on both the presence of clinical symptoms and laboratory evidence [29,40]. For the latter, two-tier serological testing is advised in most European countries, consisting of an enzyme-immunoassay (EIA) in a first step and an immunoblot for confirmation [29,31]. Since antibodies can remain present for months or even years, even after treatment, these tests don't differentiate between past and active infection, complicating diagnosis and hindering their use for follow-up of treatment [29]. For Lyme neuroborreliosis, in addition, pleocytosis and demonstration of intrathecal specific antibody production in the cerebrospinal fluid (CSF) is essential, yet can still be absent in early disease [29,34]. Polymerase Chain Reaction (PCR) can be used on a skin biopsy when there is doubt on the diagnosis or on synovial samples in case of Lyme arthritis suspicion [28,31]. On CSF, PCR is not useful due to a low sensitivity (5-17%) and similarly PCR analysis is not recommended on blood or urine [28,31].

Antibiotic treatment is recommended for all symptomatic infections, depending on the manifestation during 10 to 28 days with doxycycline (oral) or ceftriaxone (intravenous) [31]. In general, outcome after antibiotic treatment is favorable [28]. In the US, about 10% of Lyme arthritis patients develop antibiotic-refractory Lyme arthritis, in which symptoms persist despite antibiotic treatment [7]. Antibiotic prophylaxis (1 x 200 mg doxycycline) after a documented tick bite is currently not recommended [31]. A recent study in the Netherlands showed that prophylaxis led to

a significant reduction in LB risk (67%, 95% CI 31-84), yet with a high number-needed-to-treat (NNT) (51, 95% CI 29-180) [41]. The latter was also found in US studies [42]. Further evaluation and insights into how to reduce the NNT (e.g., treating persons bitten by *B. burgdorferi* s.l. positive ticks only) are needed [41].

Guidelines for the diagnosis and treatment of the different manifestations of LB for Belgium have been elaborated in 2016, and updated in 2017 [31]. These guidelines have been sent out to different healthcare professionals, including general practitioners (GPs), dermatologists, rheumatologists and pharmacists.

1.1.4 Post-treatment Lyme disease syndrome

It has been reported that about 10-15% of patients diagnosed with LB continue to report non-specific persisting symptoms after antibiotic treatment [7,35,43]. In many cases, these symptoms include fatigue, cognitive difficulties, muscle or joint pain but other symptoms such as headache, neck stiffness and paresthesias have also been described [44]. In 2006, the Infectious Diseases Society of America (IDSA) has proposed a case definition for "Post-Lyme disease syndrome (PLDS)", now often referred to as "Post-treatment Lyme disease syndrome (PTLDS)", which includes the onset of fatigue, widespread musculoskeletal pain or complaints of cognitive difficulties in patients with an initial correct diagnosis of LB treated with appropriate antibiotics [32]. According to this definition, intended for research, the symptoms should start within six months after LB diagnosis and persist for at least six months after treatment and be of such severity that, when present, they result in substantial reduction in previous levels of occupational, educational, social, or personal activities [32]. Yet, in published literature, this definition has not always been used.

To date, there is no consensus on the cause of these symptoms, but several hypotheses have been raised (autoimmunity, disturbed cytokines, deficient resistance, cognitive-behavioral mechanisms or microbiological mechanisms) [45–50]. Furthermore, it is known that several other infectious diseases such as Q fever and

Epstein-Barr virus can cause such post-infectious syndromes [51]. Randomized controlled treatment trials investigating prolonged or repeated antibiotic treatment showed insufficient evidence of the benefit of such treatments in patients with persisting symptoms attributed to LB [52–56]. In addition, these expose the patient to potential toxicity and side effects, and may induce selection of resistant bacteria. As such, symptomatic treatment is currently advised, yet controversy is existing. It should also be taken into account that these non-specific symptoms are very prevalent in the general population [57,58].

1.1.5 Chronic Lyme disease, what's in a name?

At date, there is no consensus on "chronic Lyme disease". Originally, the term referred to late LB manifestations including Lyme arthritis, ACA and chronic LNB (rare) that occur months to years after initial infection due to persistence of infection with *B. burgdorferi* s.l.. Stanek and Strle (2018) have advocated the use of this term only for this patient group [8]. However, over the past decades, the term has been used for a much broader group of patients. The latter includes patients with objective late manifestations, patients with PTLDS but also patients with chronic, subjective complaints of unknown cause, also in the absence of a documented history of LB or absence of objective physical or laboratory evidence of *B. burgdorferi* s.l. infection [35,44,59,60]. As such "chronic Lyme disease" has become a highly confusing term without a clear case definition. In general, it is important to make a clear distinction between these different groups.

1.1.6 Epidemiology

LB is widespread across the Northern -Hemisphere where tick vectors and vertebrate hosts are widely distributed. A systematic review of LB in Western Europe reported a widely varying LB incidence, ranging between 0.001/100,000 (Italy) and 464/100,000 (southern Sweden) [61]. While true heterogeneity is expected due to differences in geographical, environmental and climatic factors, in genospecies present and in human

behavior, it needs to be taken into account that comparison between countries or regions is challenging due to differences in surveillance methods, healthcare systems, case definitions and laboratory tests used [62]. An increase in the number of LB cases over the past decades has been reported in some European countries, including the Netherlands and the UK, but not in others [27,63,64]. This increase might be due to increased awareness, better recognition and improvement in diagnostics and reporting practices, but in the past years it has also been observed that *I. ricinus* ticks have been spreading to higher altitudes and latitudes, increasing the risk of infection. Causes for this expansion include changes in climate and increases in host availability [27,28,61,65].

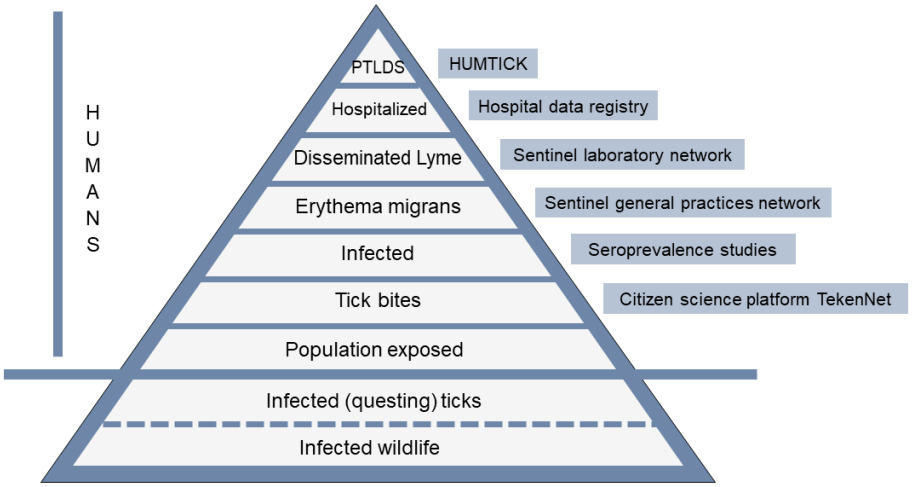
Although persons of all ages can get LB, most cases usually occur in children (5-14 years old) and adults (50-64 years old) which is possibly explained by more outdoor activities and consequently higher exposure to tick bites in these age-groups [27]. The epidemiology of LB shows a pronounced seasonal variation with a peak in June to July, yet slightlydiffering across Europe [27].

1.1.7 Surveillance in Belgium

Routine surveillance of LB in Belgium is based on different data sources (Figure 2); a network of sentinel laboratories weekly reports the number of positive serological tests for *B. burgdorferi* s.l. and the number of hospitalizations for LB is followed-up through the Minimal Clinical Data registry, a mandatory registry for all Belgian hospitals [66]. In addition, repeated prospective studies through a network of sentinel general practices (SGP) have been performed to estimate the incidence of consultations for an EM [67]. In general, no increasing trends have been observed in these data. Although the yearly number of positive serological tests has increased, mainly in 2013 and 2014, it declined again thereafter. As also the number of tests performed during that period significantly increased, this is presumably due to increased attention to the disease, both among physicians and the general population [68]. The incidence of consultations for an EM

was estimated at 83.2/100,000 persons (95% CI 73.6–92.7) in 2003–2004 and 90.2/100,000 (95% CI 80.8–100.3) in 2008–2009 [67]. The number of hospitalizations by year fluctuated between 217 and 314 for the period 2008–2018 [68].

Figure 2. Surveillance pyramid for Lyme borreliosis in Belgium



Based on Braks et al. (2011) [69]

Finally, a citizen science platform, www.tekennet.be/www.tiquesnet.be (in Dutch or French), allows to follow-up the exposure of the Belgian population to tick bites in time and space (Figure 2). Similar to the data above, yearly fluctuations are observed, which can be caused by different factors, such as weather conditions impacting tick activity, but also behavioral factors [70].

In addition to these routine surveillance systems, several research projects on LB have been carried out, not only by Sciensano but also by various universities, including seroprevalence studies [71,72]. As such, also the work included in the current thesis was set up as a research project in order to fill knowledge gaps related to LB in Belgium and more specifically PTLDS.

1.2. Other tick-borne diseases

Besides *B. burgdorferi* s.l., ticks are known to transmit several other bacteria, viruses or parasites which may cause disease in humans. Tick-borne encephalitis (TBE) is an important tick-borne infection caused by the tick-borne encephalitis virus (TBEV) which includes three pathogenic subtypes: The European subtype, the Siberian subtype and the Far Eastern subtype [73]. The European subtype, transmitted by *Ixodes ricinus,* is associated with milder disease and approximately two-third of infections are asymptomatic. If symptomatic, it mostly follows a biphasic illness with a first phase with flu-like symptoms and a second phase affecting the central nervous system (such as encephalitis or meningitis) [73]. In Europe, an increasing incidence of TBE and spread into new geographic areas has been reported in the past decades [73,74]. In 2018, the first two human cases of TBE, classified as possible and probable autochthonous, have been diagnosed in Belgium [12] and in 2020, the first three confirmed autochthonous cases have been reported [75].

Other potential tick-borne pathogens, transmitted by *Ixodes ricinus* ticks, include *Anaplasma phagocytophilum*, *Babesia* spp., *Rickettsia* spp., *Borrelia miyamotoi* and *Neoehrlichia mikurensis*, yet the amount of evidence for a causal relation between infection and disease differs by pathogen [76]. Asymptomatic infections have been described for many of these pathogens. If symptoms develop, it often concerns mild, self-limiting flu-like illness with symptoms such as fever, myalgia, arthralgia, headache or malaise. Nevertheless, more severe disease and even fatalities have been described in immunocompromised persons [76–78].

The Belgian study on ticks collected from humans in 2017 mentioned above, estimated the prevalence of these other tick-borne pathogens in ticks. Besides *B. burgdorferi* s.l., the highest prevalence was found for *R. helvetica* with 6.8% of ticks infected, yet pathogenicity of this microorganism remains unresolved with only a few case reports described [79,80]. *Babesia* spp., *A. phagocytophilum*, *B. miyamotoi* and *N.*

mikurensis were found in 1.5-2.8% of *I. ricinus* ticks [12]. *R. raoultii,* causing a syndrome characterized by scalp eschars and cervical lymphadenopathy in humans, was detected in two out of five *Dermacentor reticulatus* ticks, a species which only sporadically bites humans [12,79].

In general, compared to LB, human infections with these other tick-borne pathogens have been reported far less frequently, yet, some underdiagnoses can be expected due to the mild non-specific symptomatology, low awareness among physicians and patients but also due to a lack of routinely available diagnostic tests [78,81]. As a consequence, the public health risk and burden of these pathogens remains largely unknown. In Belgium, up to now, autochthonous human clinical cases have only been reported for anaplasmosis (10-20 probable cases annually) and TBE (see earlier) [12]. For *Babesia* spp., a retrospective study has detected specific antibodies in patients with a history of a tick bite and clinical symptoms (mainly fever) [82]. No infections or clinical cases have been reported for *B. miyamotoi*, *N. mikurensis* or *R. helvetica*.

In addition, ticks can carry and transmit more than one pathogen at a time, possibly causing co-infections. In the study in 2017, co-infections were found in 3.9% of the examined ticks [12]. In humans, co-infections can complicate the clinical picture, e.g., EM has been reported in patients infected with *N. mikurensis*, yet could have been caused by co-infection with *B. burgdorferi* s.l. 76]. Furthermore, the impact of other tick-borne infections on LB remains largely unclear, concurrent infection with *Anaplasma* spp. or *Babesia* spp. might exacerbate the course of LB [32,83]. Co-infections have been hypothesized to play a role in persisting symptoms after LB, yet there has not been convincing evidence for such an association [84].

Finally, there are also pathogens, e.g., *Bartonella* spp. and *Spiroplasma ixodetis,* that have been found in *I. ricinus* ticks, but transmission to humans through tick bites remains unconfirmed [85,86]. At day, more and more pathogens are being discovered

due to the increase in availability of molecular methods and next generation sequencing [87].

1.3. Objectives of the thesis

As highlighted in the introduction, LB is an infectious disease with a wide variety of clinical manifestations and possible persisting non-specific symptoms after treatment. To date, the burden and costs related to LB remain largely unknown. In addition, although other tick-borne pathogens are known to circulate in the Belgian tick population, little is known on the occurrence of disease in the human population.

The main objective of this thesis is to **contribute to the assessment of the burden of LB and other tick-borne diseases in Belgium**.

To achieve this objective, we designed a prospective cohort study entitled the HUMTICK study. The cohort study included LB patients and a non-LB control group. The initial study protocol and its update are described in Chapters 2 and 3 respectively.

The specific objectives of the thesis are:

- **To estimate the incidence of the different clinical manifestations of LB in Belgium**
  In Chapter 4, the most recent results on the incidence of EM according to the SGP network in Belgium are presented and a systematic review and meta-analysis are conducted on the proportional distribution of all clinical LB manifestations. The results of both are combined to allow estimation of the incidence of LB manifestations other than EM.
- **To estimate the proportion of LB patients developing PTLDS and assess possible risk factors**
  In Chapter 5, the proportion of LB patients developing PTLDS in the prospective cohort study is estimated. This is done for both patients with an erythema migrans and patients with disseminated or late LB. In addition, the occurrence of non-

specific symptoms at follow-up is compared between LB patients and a non-LB control group.

- **To estimate the costs related to LB in Belgium**

  In Chapter 6, cost data collected in the prospective cohort study are combined with other data sources, such as national data registries, in order to estimate the costs associated with LB in Belgium, taking into account both ambulatory and hospital costs. A societal perspective, which considers costs for the national healthcare insurer, the patients and their employers, is taken. Total costs, including direct medical, direct non-medical (transport and informal care) and indirect costs (productivity loss), are estimated.

- **To investigate the occurrence of other human tick-borne infections in patients with a tick bite in Belgium**

  Chapter 7 describes the results of a broad screening of EDTA-blood samples from patients with an EM (prospective cohort study) and patients with fever after a recent tick bite, using multiplex PCR techniques.

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Chapter 2

The HUMTICK study: protocol for a prospective cohort study on post-treatment Lyme disease syndrome and the disease and cost burden of Lyme borreliosis in Belgium

Adapted from:

Geebelen L, Lernout T, Kabamba-Mukadi B, Saegeman V, Sprong H, Van Gucht S, Beutels P, Speybroeck N, Tersago K. The HUMTICK study: protocol for a prospective cohort study on post-treatment Lyme disease syndrome and the disease and cost burden of Lyme borreliosis in Belgium. Archives of Public Health. 2017; 75:42.

Abstract

Background: In Belgium, different routine surveillance systems are in place to follow-up Lyme borreliosis trends. However, accurate data on the disease and monetary burden for the different clinical manifestations are lacking. Despite recommended antibiotic treatment, a proportion of Lyme patients report persisting aspecific symptoms for six months or more (e.g., fatigue, widespread musculoskeletal pain, cognitive difficulties), a syndrome now named "post-treatment Lyme disease syndrome" (PTLDS). Controversy exists on the cause, incidence and severity of PTLDS. This study aims to estimate the incidence of PTLDS in patients with Lyme borreliosis and to quantify the disease burden and economic costs associated with the different clinical manifestations of Lyme borreliosis in Belgium.

Methods: The project is a prospective cohort study in which about 600 patients with an erythema migrans and 100 patients with disseminated Lyme borreliosis will be followed up. Questionnaires, including the SF-36 vitality and pain subscale, the Cognitive Failure Questionnaire and the EQ-5D-5L, will be used to collect information on acute and persisting symptoms and the impact on quality of life. Symptom frequency and severity will be compared with self-reported pre-Lyme health status, a control group and existing Belgian population norms. Additionally, information on the associated costs and possible risk factors for the development of PTLDS will be collected.

Discussion: A study of the health burden will allow evaluation of the relative importance of Lyme borreliosis in Belgium and information on the economic cost will help to formulate cost-effective measures. There are only few prospective studies conducted estimating the incidence of PTLDS and even though discussion exists about the prevalence of subjective symptoms in the general population, a control group of non-Lyme borreliosis participants has often not been included.

Background

Lyme borreliosis is a multisystem infectious disease caused by bacteria from the *Borrelia burgdorferi* sensu lato complex. These spirochetes are transmitted by hard ticks, predominantly by *Ixodes ricinus* in Europe. Lyme borreliosis is the most prevalent arthropod-borne disease in Europe and North America [1,2]. Asymptomatic infections are frequent [3–5]. If disease develops, it can cause a wide range of symptoms. The most common clinical manifestation is an erythema migrans (EM), a red expanding rash which occurs in 60 to 95% of symptomatic infections, within days to weeks after the tick bite [6–11]. In this early disease stage, flu-like symptoms (e.g., fever, headache, fatigue, arthralgia's, myalgia's) may accompany EM or appear separately. If an infection is not treated with appropriate antibiotics, it can possibly evolve to disseminated Lyme borreliosis (weeks, months or years after the tick bite) affecting the skin, the nervous system (Lyme neuroborreliosis), the joints (arthritis) or more rarely, the heart or the eyes [1,12]. Despite adequate antibiotic treatment, it has been reported that some people still suffer from a range of non-specific symptoms after an infection: fatigue, widespread musculoskeletal pain or cognitive difficulties (e.g., memory problems, difficulties concentrating, problems finding words). If these symptoms persist for more than six months after treatment in correctly diagnosed Lyme patients and are of such severity that they influence the patient's daily life, this is referred to as post-Lyme disease syndrome [2], later named post-treatment Lyme disease syndrome (PTLDS) [13–15].

The frequency of subjective symptoms after treatment for Lyme borreliosis reported in the literature varies widely; for early Lyme borreliosis (EM) it ranges between 5 and 20% [1,13,16–19] reaching 36% if included patients present systematic symptoms as well (e.g., flu-like symptoms) [14]. A review by Koedel et al. (2015) reported that PTLDS symptoms developed in 5-54% of Lyme patients that suffered from Lyme neuroborreliosis [16]. There is controversy and concern about the prevalence of

these aspecific symptoms in the general population and although it is advised, only few studies included a non-Lyme borreliosis control group [17,19–22]. The pathogenesis of the syndrome is still unclear, different possible explanations have been suggested (e.g., persistent infection, post-infective fatigue syndrome, natural response after treatment, autoimmune mechanisms, intercurrent conditions) but no conclusions can be made [15,17,23]. Several risk factors for the development of PTLDS have been hypothesized, including dissemination of disease, delayed diagnosis and treatment, more severe symptoms at diagnosis and the elevation of immune mediators (e.g., elevated CCL19) [15,23–25].

It is thought that co-infections with other tick-borne pathogens such as *Anaplasma* spp. and *Babesia* spp. can exacerbate the clinical presentation of patients with Lyme borreliosis: patients can present with additional initial symptoms, including unexplained leukopenia, thrombocytopenia or anemia, or high-grade fever after treatment [2,26]. In the last years, other less known tick-borne pathogens have been found to circulate in Belgian ticks, amongst others, *Borrelia miyamotoi*, *Neoehrlichia mikurensis* and *Rickettsia* spp. [27–29]. It is unclear to what extent co-infections with these and other tick-borne pathogens may affect persisting symptoms after treatment of Lyme borreliosis [2,30–35].

In Belgium, it is estimated that, every year, approximately 10,000 people consult a general practitioner (GP) with an erythema migrans and 200-300 people are hospitalized due to disseminated Lyme borreliosis [36,37]. Although, up to now, no increasing trend in the Lyme borreliosis incidence has been observed (based on the long-term surveillance systems in place), the disease is assumed to represent a significant health and cost burden for the population and the health system in Belgium. This has however not yet been assessed. Lyme borreliosis and especially its possible long-lasting symptoms, even after treatment, remain a subject of controversy in Belgium, as in other parts of the world [2,38]. In order to provide some answers to this debate, this prospective study is set up.

Study objectives

The aim of the study is to evaluate the incidence of and possible risk factors for the development of post-treatment Lyme disease syndrome (PTLDS) and to estimate the disease burden and economic cost associated with the different clinical manifestations of Lyme borreliosis in Belgium.

Methods

Study design

The core of HUMTICK is a prospective cohort study with follow-up of patients with Lyme borreliosis and a non-Lyme borreliosis control group (in a 1:1 ratio). Questionnaires will be used to collect information on duration and severity of symptoms, health-related quality of life, costs and possible risk factors for the development of PTLDS. At the end of the follow-up, a case-control study will be set up within a sub-cohort of EM patients, to analyze tick-borne co-infections as a possible risk factor for the development of PTLDS. In this case-control study, cases are defined as EM patients with PTLDS and controls (in a 1:2 ratio) as patients in which EM resolves without persisting symptoms. To search for the presence of these other tick-borne diseases, a blood sample will be collected from all the patients in the EM sub-cohort at the moment of their diagnosis (acute infection). In addition to the prospective study, existing databases will be consulted to obtain supplementary data, necessary for the calculation of the full disease and cost burden of the different clinical manifestations of Lyme borreliosis.

Study population

Two sub-cohorts will be enrolled in the cohort study; one cohort consisting of patients who consult a GP with an erythema migrans and a second consisting of patients with confirmed disseminated Lyme borreliosis (e.g., neuroborreliosis, Lyme arthritis, Lyme carditis) diagnosed by a specialist physician in a hospital. A non-Lyme borreliosis control group, matched for age and gender, is added (in a 1:1 ratio) to allow comparison

between the cohorts and the general population. Children (< 18 years) and pregnant women will be excluded from the study. All participants will sign an informed consent form prior to inclusion. The case definitions for inclusion for cohort 1 and 2 are shown in Table 1.

Table 1. Case definitions for inclusion in the HUMTICK study (2016)

Cohort	Case definition
Cohort 1: EM	- Clinical presentation of EM, confirmed by a GP
Cohort 2: Confirmed disseminated Lyme borreliosis	- Positive polymerase chain reaction (PCR) or culture OR - Positive serology AND at least one clinical manifestation compatible with disseminated Lyme borreliosis (confirmed by the treating physician) [12]. [see Appendix A for the specific description of this case definition]

Participant enrollment

Participants are enrolled since June 2016 and up to August 2018 with follow-up ending no later than February 2019. For cohort 1 (EM), a network of approximately 200 GPs is currently being set up in areas in Belgium that are highly endemic for tick bites and Lyme borreliosis. All patients complying with the case definition are invited to participate, until inclusion of a sufficient sample. Patients with confirmed disseminated Lyme borreliosis (cohort 2) are enrolled through the hospitals linked to the Belgian National Reference Centers (NRC) for Lyme borreliosis: Cliniques Universitaires Saint-Luc-UCL and UZ Leuven (infectious diseases, neurology and rheumatology departments). If this is not sufficient to attain the desired sample size, other hospitals will be involved at a later stage (2017-2018). Participants of the non-Lyme borreliosis control group are selected by the patients in cohorts 1 and 2, among persons in their own environment with the same gender, comparable age (+/- 5 years) and no prior Lyme borreliosis diagnosis (self-reported, no serology performed). The participants are included in the study after validation of the criteria.

Sample size estimates and power calculation

For the sample size calculation of **cohort 1**, we assume that 12.5% of EM patients will develop PTLDS (mean of 5-20% [1,13,16–19]) in comparison with 5% [19] of the non-Lyme controls developing the same symptoms. For the sample size calculation of **cohort 2**, we assume 20% of disseminated Lyme patients developing PTLDS. With an alpha of 0.05, a power of 90 and a cohort-controls ratio of 1:1, this means that we need 285 EM patients in cohort 1 and 93 disseminated Lyme patients in cohort 2 (2 sided-test) to allow detection of a risk ratio (RR) of respectively 2.5 and 4.

However, to analyze the association between PTLDS and co-infections as a risk factor in the **case-control** study (set up within cohort 1), we need 74 cases of PTLDS and 148 controls based on an alpha of 0.05, a power of 0.80, a case-controls ratio of 1:2 and the assumption of a less than medium effect size of 0.4 (2-sided test). This means that, if 12.5% of the EM patients develop PTLDS, we need to extend **cohort 1** to 592 EM patients in order to have 74 cases in the case-control study**.** Taking into account a reasonable loss to follow-up (± 25% over 2 years), 780 EM patients and 120 disseminated Lyme patients need to be included in the study.

Data collection procedures

Data collection will start when the patient is diagnosed (T0) with Lyme borreliosis at the consultation with the GP (cohort 1) or in the hospital (cohort 2). During this consultation, the first part of the first paper questionnaire is filled in together with the treating physician (questions on comorbidity, acute symptoms, diagnosis and treatment). The second part of the questionnaire can be filled in by the patients themselves after the consultation (questions on socio-demographic parameters, general health before Lyme borreliosis, tick bite exposure, acute symptoms and costs). The patients' follow-up questionnaires are completed online (or on paper if preferred by the patient) at different points in time: after 1 month, 3 months, 6 months, 12 months and 24 months. The period of patient follow-up depends on the moment of enrollment and will last maximum 24 months (patients enrolled in early study phase)

and minimum 6 months (patients enrolled in final phase of enrollment). Control persons will be requested to fill in a questionnaire at inclusion, after six months and after one year. The blood sample for the patients of cohort 1 (EM) will be collected at T0.

Post-treatment Lyme disease syndrome (PTLDS)

PTLDS is defined (in line with the case definition for post-Lyme disease syndrome proposed by the Infectious Diseases Society of America (IDSA) [2,13]) as the new onset of fatigue, widespread musculoskeletal pain and/or cognitive difficulties in patients diagnosed with and treated for Lyme borreliosis. Symptoms should occur within six months after the diagnosis, be present for more than six months (continuous or relapsing) and impact the patient's daily life functioning (quality of life). Patients will be excluded if the symptoms are not new or when there is another explanatory cause for their symptoms.

To apply this case definition, the first patient questionnaire (T0) will assess the presence and severity of the subjective symptoms, as well as the impact on the patient's life, before the onset of their Lyme borreliosis (during their "pre-Lyme" general health status). The post-treatment presence, severity and impact of the subjective symptoms will be assessed in the same way, in the different follow-up questionnaires (from 3 months onwards). This will allow comparison between the pre- and post-Lyme health status of the patient.

In order to assess the severity and impact more accurately, validated questionnaires will be used:

- The SF-36 is a self-report 36-item health survey (8 subscales) which has been used widely and was shown to have good reliability and validity [39–41]. In our study two subscales are used; the SF-36 vitality subscale (4 items) to assess

fatigue and the SF-36 bodily pain subscale (2 items) to assess widespread musculoskeletal pain.

- The Cognitive Failure Questionnaire (CFQ), a 25-item questionnaire of which psychometric properties have been proven to be good, is used to evaluate cognitive difficulties [42,43].
- The EQ-5D-5L, a standardized non-disease specific instrument to evaluate the health-related quality of life, developed by the EuroQol group association [44–46], is added to each questionnaire to assess the impact of the symptoms on the patient's quality of life.
- The Global Activity Limitation Index (GALI), a single item instrument, is added to the questionnaires from 6 months onwards since it measures the presence of long-lasting (≥ 6 months) health related activity limitations [47–49].

The same questionnaires will be used for people from the non-Lyme borreliosis control group, to allow analysis of the incidence of the same symptoms in the general population. Furthermore, currently Belgian population norms exist for the SF-36 vitality, SF-36 bodily pain, the EQ-5D-5L and the GALI, CFQ norms are only available for the Dutch population [50–54].

Finally, the GPs of the patients in cohort 1 (EM) will fill in an online follow-up questionnaire, based on the patient's medical file, after six and twelve months. Questions are asked about possible supplementary consultations, additional diagnoses, new treatments prescribed and laboratory results if requested. This will allow comparison between the patient's and GP's perspectives on the subjective symptoms.

Risk factors for PTLDS

To identify possible risk factors for the development of PTLDS, information on comorbidity, tick bite exposure, severity and duration of symptoms presented, prescribed treatment (type, duration) as well as socio-demographic variables (age, sex, education and employment status) will be collected during the complete study. The

blood samples collected at T0 from EM patients will be tested, by means of a multiplex PCR, for the presence of *Anaplasma* spp.*, Rickettsia* spp.*, Neoehrlichia mikurensis, Borrelia miyamotoi* and *Babesia* spp. at the Laboratory for Zoonosis and Environmental Microbiology (RIVM), the Netherlands [55].

Disease burden

The Burden of disease will be expressed in Disability-Adjusted Life Years (DALYs), a summary health measure comprising both mortality and morbidity. DALYs measure the healthy life years lost due to illness and equal the sum of the years lived with disability (YLDs) and the years of life lost due to premature mortality (YLLs). The YLDs are calculated based on the occurrence, severity (disability weights (DW)) and duration of the disease health states [56–58]. An incidence approach will be used for the occurrence of the different clinical manifestations of Lyme borreliosis, where the incidences of EM and disseminated Lyme will be estimated based on existing literature and surveillance systems currently in place in Belgium (e.g., sentinel network of general practitioners, network of sentinel laboratories and minimal clinical data) [36,37]. The cohort study will provide an estimate of the incidence of PTLDS, of the symptom durations for the different clinical manifestations as well as estimates of the group specific disability weights. The latter will be derived from the participants' EQ-5D-5L responses: following the EQ-5D-5L user guide, the EQ-5D-5L scores will first be converted into utilities using a pre-existing preference valuation set for EQ-5D health states of the Belgian (Flemish) adult population [59–61]. The resulting utility, a value between 0 (death) and 1 (full health), will then be transformed to DWs by subtracting it from the EQ-5D population averages for the same sex and age-group [52,62,63]. Since Lyme related mortality is exceptional and no Lyme related deaths have been reported in Belgium between 1998 and 2014 (only data available), YLLs will equal zero.

Costs

To minimize recall bias between two consecutive questionnaires (recall periods up to 12 months at the end of the follow-up period), patients are invited to keep a cost diary during the complete study period. The patients' questionnaires assess direct and indirect non-medical costs (e.g., travel costs, absence from work, paid help) and provide information on direct medical costs related to medication use, consultations and hospitalizations (e.g., how many visits to a GP/specialist/…, use of over the counter medication). Additional details on some of these direct medical costs, which can't be provided by the patients themselves, are collected through the follow-up questionnaires completed by the GPs (e.g., information on the laboratory tests). The standard unit costs (the price of medication, a consultation, a laboratory test, a hospitalization) will be obtained from official sources (e.g., Belgian Centre for Pharmacotherapeutic information (BCPI), National Institute for Health and Disability Insurance (RIZIV)). Supplementary data on hospitalization costs will be collected through the minimal hospital data (MZG), minimal financial data (MFG) and Belgian health insurance databases.

Statistical analysis

All analyses will be performed in R [64]. A conditional log-binomial regression model with adjustment for comorbid illness will be used to compare the development of the non-specific disease symptoms between the different cohorts and the "matched" non-Lyme borreliosis control group in order to calculate the incidence of PTLDS in patients with Lyme borreliosis [65]. Within cohort 1 & 2, a multivariate log-binomial regression model will be used to calculate exposure risk ratio (RR) of the risk factors for development of PTLDS. For the case-control, the odds ratio (OR) determination for tick-borne co-infections as a risk factor will be done using a multivariate logistic regression. The multivariate models will include adjustment for potential confounding variables (age, gender and comorbid illness).

The burden of disease will be estimated in collaboration with the "Institut de recherche santé et société (IRSS) Université catholique de Louvain (UCL)" using the DALY package [56–58,66]. The total costs (mean, median & confidence intervals) for each of the different clinical manifestations, including PTLDS, will be calculated by combining the different data sources. The cost analysis will be carried out in collaboration with the Centre for Health Economics Research & Modeling Infectious Diseases (CHERMID), University of Antwerp (UA).

Discussion

Lyme borreliosis has been an increasingly "hot topic" in Belgium and the rest of the world, with uncertainties and intensive debates on the burden of the disease and on possible long-lasting ("chronic") symptoms.

The incidence of PTLDS has been estimated in previous studies, but the reported results vary widely. This is likely related to differences in the inclusion criteria and PTLDS case definitions used, as well as to the variation among study designs [15]. The strength of the presented study lies in the combination of different methodologies which aim at collecting objective data with regard to PTLDS: the prospective study design allows collecting detailed information throughout the complete disease progression and minimizes recall bias; the inclusion of a non-Lyme borreliosis control group allows controlling for the background prevalence of aspecific symptoms in the general population; through the use of validated questionnaires the severity of the aspecific symptoms will be assessed (and afterwards compared) accurately. The availability of Belgian population norms for some of the validated questionnaires is an additional benefit. Furthermore, the use of parallel questionnaires for the patients and their GPs allows comparison between the different perspectives on the persistent aspecific symptoms as well as to obtain detailed information on possible risk factors (e.g., comorbidity, diagnosis, treatment, complications). By looking at both the prevalence

and the severity of the symptoms occurring with PTLDS, as well as the impact on the patient's life, we will be able to assess all components of the PTLDS case definition [13]. Different possible risk factors will be assessed through the patient questionnaires and the analyses of other tick-borne co-infections (in EM patient blood samples) allows collection of innovative data on the importance of co-infections on the clinical presentation and progression of Lyme borreliosis. A better comprehension of this syndrome and its risk factors will allow informing healthcare providers and patients about what to expect after treatment and, if possible, to early identify those patients at increased risk.

The estimation of the burden of disease, quantified by a composite health measure, can be used to compare and prioritize between the different clinical manifestations of Lyme borreliosis but also between different diseases and health interventions, in order to set public health priorities [67]. Some European projects assessed the burden of different communicable diseases but they did not include Lyme borreliosis [67,68]. A study in the Netherlands did estimate the disease burden for Lyme borreliosis and found that the majority of the substantial burden is caused by persisting symptoms [69]. The additional benefit of our study for the burden estimation is again its prospective study design. In the end, the results will be used to inform the general public, patients associations, policymakers and healthcare providers on the actual disease burden related to the different stadia of Lyme borreliosis in Belgium.

A few European studies have examined the costs associated with Lyme borreliosis but often only part of the costs were included (e.g., costs of Lyme neuroborreliosis in Sweden [70], cost of testing [71] and of hospital care in Germany [72]). One study in Scotland included both direct and indirect costs for early and late Lyme borreliosis patients and follow-up costs [73]. These studies didn't include the specific supplementary cost for patients with PTLDS although it is expected to contribute significantly to the overall costs of Lyme borreliosis. A recent American study showed that PTLDS-related diagnoses are associated with notably higher costs and healthcare

utilization ($3,798 higher costs and 66% more outpatient visits) as compared to Lyme borreliosis without PTLDS-related diagnoses [21,74]. Through the inclusion of different costs (both direct and indirect, medical and non-medical) for both the patients and the healthcare system, this study will provide an overall view of the costs associated with Lyme borreliosis, its diagnosis, treatment, follow-up, etc. The results could be used in future cost-effectiveness analyses of potential interventions.

A first possible limitation of the study is the workload for the patients at the moment of inclusion (long questionnaire) which might keep them from participating. However, in the context of the current attention for Lyme borreliosis in the media and the debate on existing persisting symptoms, we believe we will find sufficient patients willing to participate in the study. Secondly, as prescribed in the Belgian guidelines for the diagnosis and treatment of Lyme borreliosis, diagnosis of an EM is solely based on clinical symptoms. This implies that the inclusion of EM patients in our study is dependent on correct EM recognition by the participating GPs. As a reminder, the clinical signs of an EM, possible differential diagnoses and corresponding pictures are provided to the participating GPs at the start of the study. The diagnosis of disseminated Lyme borreliosis is based on both clinical symptoms recognized by specialists and laboratory testing. Third, due to project time restrictions, we will only be able to follow up patients during a maximum of two years. It would have been interesting to follow up patients for a longer period; many patients and patient associations indeed have questions about symptoms after a longer period after treatment. However, a longer study period is likely to induce an important loss to follow-up over time. Finally, the study will not allow the collection of data with regard to a group of patients who attribute their persisting aspecific symptoms (fatigue, musculoskeletal pains and cognitive disorders) to Lyme borreliosis, but without having a confirmed diagnosis.

In conclusion, through its multidisciplinary approach, the HUMTICK prospective cohort study allows addressing multiple relevant research questions with regard to Lyme borreliosis; the enrollment of both EM patients (early localized Lyme) and disseminated Lyme borreliosis patients, together with the follow-up over time, will not only allow to estimate the incidence of PTLDS but also to estimate the disease burden and cost associated with the different manifestations of Lyme borreliosis, specifically in Belgium. In addition, the study design allows simultaneous evaluation of risk factors for the development of PTLDS and will so improve the understanding of the evolution of the different clinical manifestations of Lyme borreliosis after treatment.

Ethics, consent and permissions

The study will be conducted in compliance with the principles of the Declaration of Helsinki (2008) and all of the applicable regulatory requirements. *Ethical approvals* were obtained from the "Comité d'Ethique Hospitalo-Facultaire Saint-Luc UCL" (ref.: 2016/13AVR/166) and the "Commissie Medische Ethiek UZ KU Leuven" (ref.: S59379/ B403201628482). *Informed consent* will be obtained from all study participants. The study has been approved by the *Commission to Protect the Personal Privacy* (Beraadslaging Nr. 16/038, reference: SCSZG/16/144).

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Appendix A

Specific description of the case definitions for confirmed cases of disseminated Lyme borreliosis which will be included in the HUMTICK study:

Adapted from CDC and EUCALB case definitions* [1-2]

- Isolation or positive PCR from tissue or body fluid

OR

- Positive serology (using the two-tier ELISA and Western Blot)

  AND

  at least one of the following clinical manifestations corresponding with disseminated Lyme borreliosis, when an alternate explanation is not found:

  - **Skin manifestation:**

    Multiple erythema migrans or acrodermatitis chronica atrophicans.

  - **Musculoskeletal system (Lyme arthritis):**

    Recurrent episodes (weeks or months) of objective joint swelling in one (commonly the knee) or a few joints, sometimes followed by chronic arthritis in one or a few joints. The following manifestations are not considered for inclusion: chronic progressive arthritis not preceded by brief attacks, chronic symmetrical polyarthritis, and arthralgia, myalgia, or fibromyalgia syndromes alone.

  - **Nervous system (neuroborreliosis):**

    Any of the following neurological symptoms (alone or in combination): lymphocytic meningitis; cranial neuritis, particularly facial palsy (uni- or bilateral); radiculitic pain; or, rarely encephalomyelitis, confirmed by demonstration of antibody production against *B. burgdorferi* in the cerebrospinal fluid (CSF), evidenced by a higher titer of antibody in CSF than in serum (positive antibody index). Headache, fatigue, paresthesia, or mildly stiff neck alone, are not considered for inclusion.

- **Cardiovascular system (carditis):**

  Acute onset of high-grade (2nd-degree or 3rd-degree) atrioventricular conduction defects that resolve in days to weeks and are sometimes associated with myocarditis. Palpitations, bradycardia, bundle branch block, or myocarditis alone are not considered for inclusion.

* Belgium is an area endemic for Lyme Borreliosis; therefore, all included patients fulfill the criteria of exposure to *B. burgdorferi.*

References in the appendix

1. Stanek G, Fingerle V, Hunfeld K-P, Jaulhac B, Kaiser R, Krause A, et al. Lyme borreliosis: Clinical case definitions for diagnosis and management in Europe. Clin Microbiol Infect. 2011;17:69–79.

2. Centers for Disease Control and Prevention. Lyme Disease (Borrelia burgdorferi) 2011 Case Definition. https://ndc.services.cdc.gov/case-definitions/lyme-disease-2011/. Accessed 16 Nov 2021.

Chapter 3

Adaptations to the initial study protocol

Introduction

The initial study protocol for the HUMTICK prospective cohort study (**Chapter 2**) was written at the start of the patient inclusion and was submitted for publication on 1/12/2016. When conducting the study in practice, some difficulties were encountered that required revision of some of the proposed approaches, to allow the objectives to be achieved. Because fewer participants than anticipated could be included during the first year of the study, changes were made to the inclusion methods to increase the number of participants. In addition, some practical difficulties implicated changes in the data collection methods during follow-up. The need for these adjustments provides important insights for future prospective research in similar settings.

Participant enrollment

Participant recruitment was extended up to December 2019 instead of August 2018 as initially foreseen in the study protocol. The network of GPs, set up to include patients with an EM (cohort 1) gradually expanded during the study period. In the first year (2016), GPs from municipalities with the highest LB incidences were invited, leading to a network of 50-100 GPs at the beginning and end of the tick season respectively. As this was less than the 200 GPs foreseen, in 2017 and 2018, more GPs were invited, in larger areas in the provinces with the highest LB incidences, leading to about 200 participating GPs from the start of the 2018 tick season onwards. Every year, a reminder was sent to the participating GPs.

Patients with confirmed disseminated Lyme borreliosis (cohort 2) were, as of 2017, not only included through Cliniques Universitaires Saint-Luc-UCL and UZ Leuven, but also through six additional hospitals/medical centers; Ziekenhuis Oost-Limburg (ZOL), ReumaClinic Genk, CHU UCL Namur (site Godinne), Centre Hospitalier Universitaire de Liège, Clinique Saint-Pierre Ottignies (CSPO) and Centre Hospitalier Régional de Namur (CHRN). Depending on the hospital, the departments of infectious diseases, neurology and rheumatology were all involved, or only some of them.

As not all patients included in cohorts 1 and 2 were able to provide a control person selected from their own environment, those for whom it was possible, were asked to include multiple controls (max. three). Furthermore, additional controls were recruited by asking participating controls to also include persons from their environment, based on the same requirements (same gender, age +/- 5 years and no prior LB). Finally, at the end of the inclusion, additional persons in gender and age categories lacking controls were invited through online invitations. All control persons fulfilling the inclusion criteria were included in the analysis, hence there was no more 1:1 matching. For future research, it would be useful to use more comprehensive methods allowing to include sufficient control persons.

Despite the prolongation of the study, less patients than foreseen could be included (Table 1). Compared to 2016, the years 2017 and 2018, but mainly 2019, were years with a lower number of tick bites and less LB cases in Belgium [1, 2]. In addition, it is also possible that there was a decrease in the motivation of GPs over the years.

Table 1. Patients recruited, informed consent received

	Cohort 1: EM	Cohort 2: Disseminated LB	Control group	Other TB infections
2016	32	1	11	3
2017	45	13	33	5
2018	50	8	35	5
2019	14	7	78	3
Total[a]	**141**	**29**	**157**	**16**

EM: erythema migrans; LB: Lyme borreliosis; TB: tick-borne

[a] Included by GP, hospital or other participants, not necessarily fulfilling all inclusion criteria, the latter were further checked and some additional patients were excluded.

Because of the smaller sample size, for the analysis of other tick-borne pathogens, blood samples of all EM patients could be tested by means of multiplex PCR, hence no case-control study was set up within only a sub-cohort of EM patients, as initially foreseen.

Data collection procedure

In order to comply with privacy regulations, patients had to send the first questionnaire (GP and patient part) and their informed consent by post to Sciensano themselves. However, it seemed that several patients, although they agreed in participate to the study at the GP consultation and filled in the informed consent, did not send their documents in the end. It might be possible that they forgot to send it, that some documents were lost by the post or that patients changed their mind about wanting to participate after the consultation. Except for contacting the GP to ask if they could re-contact the patient, which helped in a number of cases, no other solution for this problem was found during the study period. In the end, the informed consent was never received for a substantial part of patients, namely 39 EM patients (not included in Table 1). The possibility of this problem occurring and potential solutions should be considered for future studies in similar settings.

Finally, it was not feasible to have the GPs fill in the online follow-up questionnaires due to technical problems. These questionnaires had to be filled in through a specific tool (e-forms) in the GP's medical software programs which allowed recoding of the patients' ID by a third party, again necessary to comply with privacy regulations. However, this tool was not yet in routine use at the time our study started and there were many technical problems with the rollout of the questionnaires in practice. In the end, too few GPs were able to fill in the forms and the results could not be used. No other solution was found, hence there were only questionnaires filled in by the patients themselves at follow-up (online or paper version). Although such e-forms are currently routinely used in the context of the COVID-19 epidemic, it needs to be taken into account that technical problems may occur as GPs use a wide variety of software packages, all of which must correctly implement and maintain the e-form.

Chapter 4

Combining primary care surveillance and a meta-analysis to estimate the incidence of the clinical manifestations of Lyme borreliosis in Belgium, 2015–2017

Adapted from:

Geebelen L, Van Cauteren D, Devleesschauwer B, Moreels S, Tersago K, Van Oyen H, Speybroeck N, Lernout T. Combining primary care surveillance and a meta-analysis to estimate the incidence of the clinical manifestations of Lyme borreliosis in Belgium, 2015–2017. Ticks and Tick-borne Diseases. 2019;10:598–605.

Abstract

Lyme borreliosis (LB) is an important tick-borne disease which can cause a broad range of symptoms mainly affecting the skin, the nervous system and the joints. This study aims to estimate the incidence of the different clinical manifestations of LB in Belgium. The incidence of erythema migrans (EM) was estimated through the network of sentinel general practices at 97.6/100,000 inhabitants (uncertainty interval [UI] 82.0–113.0) for the period 2015 to 2017. This result was used to estimate the incidence of other LB manifestations based on their proportional distribution (ratios) to EM reported in the neighboring countries of Belgium. To estimate these ratios, we performed a systematic review of studies published between February 1, 2008 and January 31, 2018 and pooled the results using a random effects meta-analysis. Six studies were retained in the systematic review, and the meta-analysis estimated the occurrence ratios for Lyme neuroborreliosis/EM, Lyme arthritis/EM and other manifestations/EM at 0.024 (95% confidence interval [CI] 0.016–0.037), 0.022 (95% CI 0.020–0.024) and 0.014 (95% CI 0.012–0.016) respectively. Applying these ratios to the EM incidence in Belgium resulted in an incidence estimation of 2.4/100,000 inhabitants (95% UI 1.5–3.7) for Lyme neuroborreliosis, 2.1/100,000 (95% UI 1.7–2.6) for Lyme arthritis and 1.4/100,000 (95% UI 1.1–1.7) for other less frequent manifestations. Some of these LB manifestations, other than EM, are more severe, hence these estimates are essential to assess the health burden and economic cost of LB which would be highly relevant for patients, healthcare providers and policymakers. As both over- and underestimation of different clinical LB manifestations remain possible due to characteristics of the primary surveillance systems and the disease itself, future studies to validate these estimates would be of great value.

Introduction

Lyme borreliosis (LB) is caused by bacteria of the *Borrelia burgdorferi* sensu lato (s.l.) complex and is the most prevalent tick-borne disease in Europe and North America. It is a multisystem infectious disease, which can generate a wide range of clinical manifestations [1,2]. An early localized infection mainly manifests as erythema migrans (EM), a red expanding skin lesion at the site of the tick bite. EM is the most common manifestation of LB but it is not always present. If untreated, disseminated infection can occur in an early or late stage of the disease, causing more severe manifestations of which multiple EM, Lyme neuroborreliosis (LNB) and Lyme arthritis (LA) are the most frequent ones. Other less frequent early or late manifestations are borrelial lymphocytoma, Lyme carditis, ocular manifestations and acrodermatitis chronica atrophicans (ACA) [1,2].

In order to assess the health burden and costs of LB, data on the incidence of all the different clinical manifestations are required [3–6]. In Belgium, the incidence of consultations for an EM is estimated through prospective studies in a network of sentinel general practices (SGP), which are repeated over time (2003-2004, 2008-2009 and since 2015 onwards) [7]. In addition, a network of sentinel laboratories weekly reports the number of positive serological tests for *B. burgdorferi* s.l., while hospitals monitor the yearly number of hospitalizations for LB [8,9]. Yet, these data do not allow an accurate estimation of the incidence of disseminated and late LB manifestations in Belgium. This is because clinical information is missing for the laboratory results and a positive serology alone does not allow to distinguish between active and past infection [10], and the proportion of patients that are hospitalized is unknown. Furthermore, it is difficult to compare reported incidences between countries, due to differences in surveillance methods, healthcare systems, case definitions, and laboratory tests used [11,12]. Nevertheless, comparing the proportional occurrence of the different clinical manifestations between countries with a similar level of healthcare could be possible,

taking into account that different disease manifestations are associated with different pathogenic genospecies of the *B. burgdorferi* s.l. complex and that their prevalence differs geographically [2,13–16]. In the United States, LB is predominantly caused by *B. burgdorferi* sensu stricto*,* a genospecies preferentially associated with LA. In Europe, at least five genospecies are known to be pathogenic, namely *Borrelia afzelii*, *Borrelia garinii, B. burgdorferi* sensu stricto, *Borrelia spielmanii* and *Borrelia bavariensis. B. afzelii* and *B. garinii* are most frequently detected and more often associated with ACA and LNB, respectively [1,13,15,17,18]. Although the prevalence of these genospecies in ticks may vary within Europe, a recent systematic review by Strnad et al. (2017) found no significant difference in the proportional representation of the genospecies between Western Europe (Belgium, France, Luxembourg, and the Netherlands) and Central Europe (such as Germany, Austria, Switzerland) [16].

The main objective of this study is to estimate the incidence of the different clinical manifestations of LB in Belgium. In a first step, the most recent results on the incidence of EM according to the SGP network are presented. Secondly, a systematic review and meta-analysis on the proportional distribution of the different clinical manifestations of LB in the neighboring countries of Belgium is conducted in order to estimate the ratios of other LB manifestations to EM. Finally, the incidence of LB manifestations other than EM in Belgium is estimated by a data synthesis integrating both steps.

Methods

Incidence of erythema migrans

The Belgian network of SGP is a nationwide network of general practitioner (GP) practices representative of the Belgian GPs, in which each practice comprises one or multiple participating physicians, who voluntarily transmit data for different health problems [19]. Cases of EM, defined as a skin lesion (or multiple skin lesions) that expands over a period of days to weeks, with a diameter of minimum 5 cm, and often

with partial central clearing [10,20], reported by the network between 2015-2017 were included in this study. For each case a questionnaire is completed containing patient characteristics and specific questions about the tick bite (if recalled), a possible prescription of serological tests and treatment. To estimate the population covered by the network, the number of inhabitants per active GP (defined as having at least 500 contact patients/year based on health insurance data) was calculated in each of the 43 districts in Belgium and further multiplied by the number of sentinel practices (minimum population coverage) or the number of sentinel GPs (maximum population coverage). The average population covered by the network for the period 2015-2017 was estimated at 147,749 inhabitants (1.3% of the Belgian population). EM incidence estimates were generated at national level, for the three Belgian regions – i.e., the Brussels Capital, Flemish and Walloon Region and per province. Uncertainty in the estimates was quantified and propagated using 10,000 Monte Carlo simulations following a uniform distribution defined by the minimum and maximum incidence estimate (based on the maximum and minimum population coverage estimate respectively). Results were summarized by the mean and a 95% uncertainty interval (UI) defined as the 2.5^{th} and 97.5^{th} percentile of the uncertainty distribution. All calculations were done in R version 3.5.0 [21].

Systematic review and meta-analysis

Search strategy and study selection

A systematic review on the proportional occurrence of the different clinical manifestations of LB was conducted and reported following the PRISMA guidelines [22] (Appendix A). Literature from Belgium and its neighboring countries was searched through PubMed and Embase with the following search terms (in February 2018): (Lyme disease) AND (epidemiology OR incidence OR prevalence OR clinical manifestation*) AND (Belgium OR France OR Netherlands OR Germany OR Luxembourg OR United Kingdom) NOT ((animals) NOT ((animals) AND humans)). The searches included MeSH terms in PubMed and Emtree terms in Embase as well as free text

searches. The searches were limited to publication dates between 01/02/2008 and 31/01/2018 and to English, Dutch, French or German language. Details of the search strategies can be found in Appendix B (Table B1). Furthermore, grey literature, more specifically the websites of the national public health institutes of the included countries, was searched (Appendix B, Table B2).

All manuscripts retrieved through this search strategy were first screened based on title and abstract. Next, full texts of potentially eligible papers were obtained and assessed through the use of an inclusion criteria checklist by two authors (LG and TL) independently. Papers were included if they fulfilled all the following criteria: (1) description of observational study results or surveillance data from the most recent ten years; (2) quantitative reporting on the incidence or proportional distribution of at least the three most frequent clinical manifestations of LB (EM, LNB and LA) with or without reporting on the other manifestations; (3) covering the general LB population and (4) using case definitions in line with those published by Stanek et al. (2011), as these are used for clinical diagnosis in Europe. More specifically, EM had to be a purely clinical diagnosis and other LB manifestations had to be confirmed with laboratory testing. As a consequence, studies on subgroups (e.g., risk groups, hospitalized patients, persons with a tick bite), case reports and laboratory-based studies only were excluded. Only data on confirmed cases were retained.

Quality assessment

The quality assessment of included studies focused on adaptations made to the European case definitions [10], verification of the compliance of the reported cases with the definition and other case validation methods applied. In addition, epidemiological data characteristics were compared and possible limitations of the studies, provided by the authors themselves or appraised by the reviewers, were assessed.

Data extraction

For the studies that met the inclusion criteria, the following data were extracted into a data extraction form by two authors (LG and TL) independently: country (and region), study design, reporting entity (who, coverage (sentinel/comprehensive) and type (voluntary/mandatory)), inclusion period, study population, inclusion criteria, data collection methods, total sample size and the frequency of the clinical manifestations. Additional information on the study quality (case definitions used, validity measures taken and possible limitations) was recorded. If important data were missing or unclear, the original author of the paper was contacted.

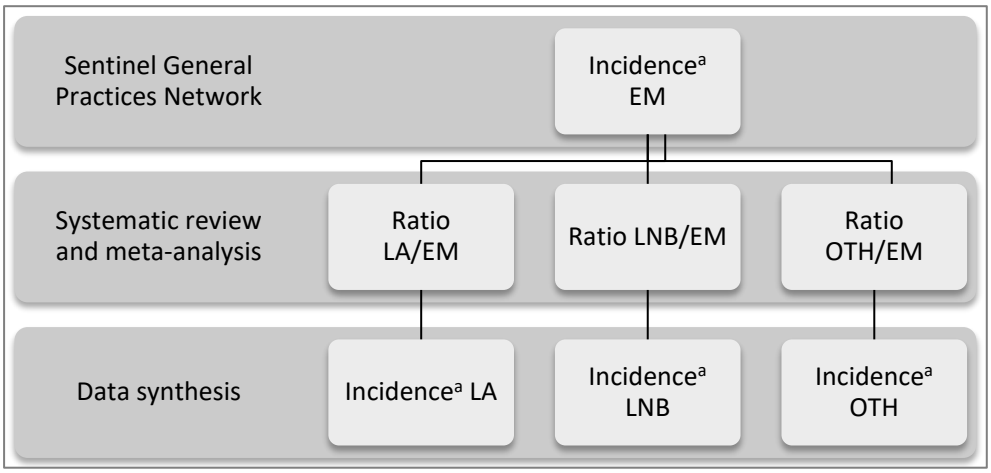
Meta-analysis

We generated pooled estimates of the ratios of LA, LNB, and "other manifestations" of LB to the number of EM manifestations, using random effects meta-analyses for log-transformed rates [23]. Borrelial lymphocytoma, ACA, Lyme carditis and, if available, ocular manifestations were combined into the group of "other manifestations" as only very few cases were found in the different studies. The influence of exclusion of studies based on mandatory surveillance was explored in an alternative scenario, as differences in surveillance methods could influence the results. All meta-analyses were performed using the metafor package for R version 3.5.0 [21,24].

Data synthesis

The result from the incidence estimation of EM through the Belgian SGP network was combined with the results of the meta-analysis on the proportional occurrence of clinical manifestations of LB in order to calculate the total incidence of LB in Belgium, including disseminated and late manifestations (Figure 1). Uncertainty, quantified as uniform distributions for incidence estimates and log-normal distributions for ratios, was propagated using 10,000 Monte Carlo simulations. All calculations were performed in R version 3.5.0 [21].

Figure 1. Overview of methods used to quantify the incidence of clinical manifestations of Lyme borreliosis



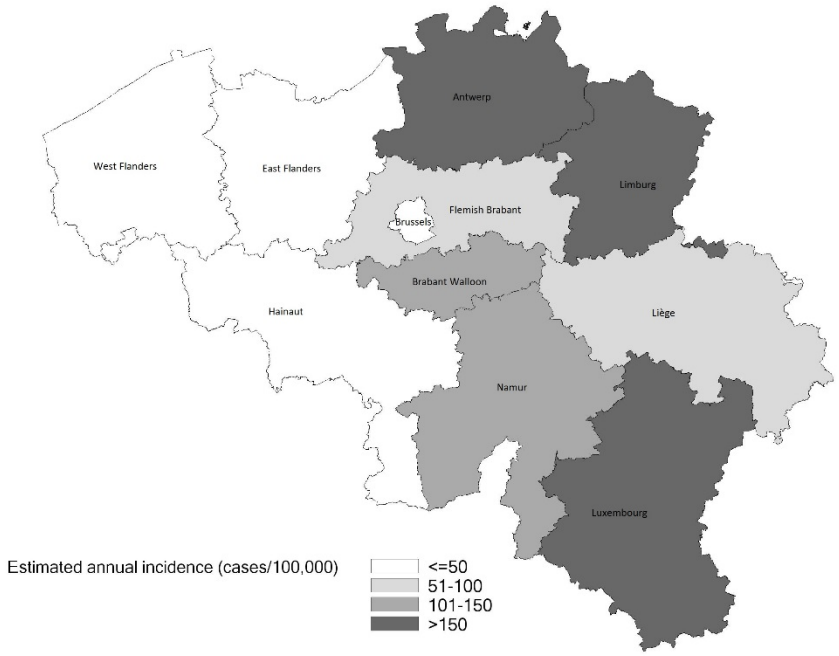
EM: erythema migrans; LA: Lyme arthritis; LNB: Lyme neuroborreliosis; OTH: other manifestations
[a] Incidence in Belgium.

Results

Incidence of erythema migrans

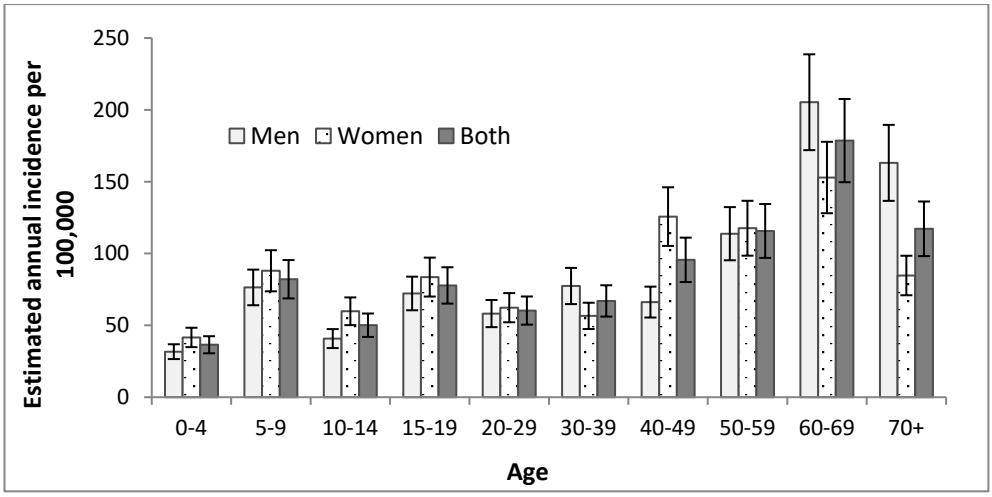
In the period 2015-2017, 420 EM cases were reported by the SGP network, resulting in an estimated annual incidence of 97.6 EM cases/100,000 inhabitants (95% UI 82.0–113.0). The estimated annual incidence corresponded to 98.0 cases/100,000 (UI 81.8–114.2) in 2015, 106.1 cases/100,000 (95% UI 90.1–122.2) in 2016 and 88.5 cases/100,000 (95% UI 74.3–102.8) in 2017. Figure 2 shows the annual incidence for each province in Belgium. The estimated annual incidence ranged from 30.9 cases/100,000 (95% UI 21.4–40.3) in East Flanders to 390.9/100,000 (95% UI 329.7–451.9) in Limburg. Regionally the estimated annual incidence was 126.0 cases/100,000 (95% UI 101.0–150.9) in the Flemish region, 74.2 cases/100,000 (95% UI 68.3–80.1) in the Walloon region and 34.0 cases/100,000 (95% UI 28.4–39.6) in the Brussels Capital region.

Figure 2. Estimated annual incidence of erythema migrans (cases/100,000 inhabitants) per province, Sentinel General Practices network, Belgium, 2015-2017



Women represented 49.6% of the cases and the incidence peaked in the 60-69 years age group, especially in men (Figure 3). A typical seasonality with a peak during the summer period was also observed, with 60.5% of the cases reported between June and August.

Figure 3. Estimated annual incidence of erythema migrans (cases/100,000 inhabitants) by age and gender, Sentinel General Practices network, Belgium, 2015-2017

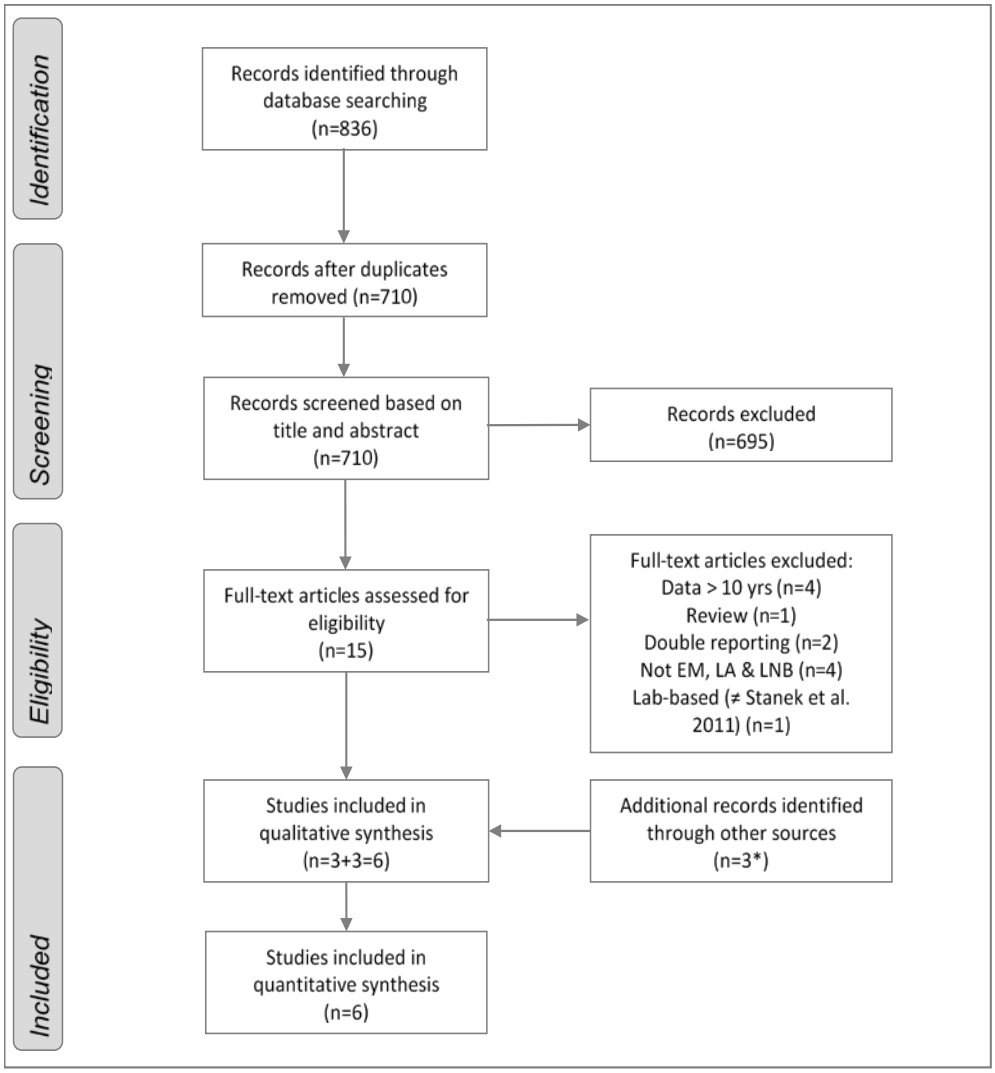


Systematic review and meta-analysis

Literature search

We identified 836 citations of which, after deletion of duplicates, 710 were screened based on title and abstract. Among these, 15 were potentially eligible epidemiological studies or reports of surveillance data for which full texts were obtained. A total of three studies met the inclusion criteria [14,25,26]. Studies were excluded based on: double reporting [5,14], data (partly) older than 2008 [27–30], review article [31], not reporting on EM, LA and LNB [7,8,32,33] and not in line with the European case definitions [34]. In addition, data from two surveillance reports were included through the search of public health websites of the concerned countries [35,36]. Since one report described two surveillance systems, three data sets could be added. Duplicate data were excluded. A PRISMA flowchart is provided in Figure 4. No studies or reports from Luxembourg were found and none of the UK studies fulfilled the inclusion criteria since surveillance in the UK is based on laboratory confirmed cases only.

Figure 4. Study selection flowchart



* One record provided data from two studies, included as two studies.

Study characteristics

The selected data were from the Netherlands (n=1) [14], Germany (n=3) [26,35] and France (n=2) [25,36]. An overview of study characteristics is presented in Appendix C (Table C1). LB cases were reported by GPs at a national level in two of the studies included – i.e., retrospectively through a survey of GPs in the Netherlands [14] and

prospectively through weekly reporting by the *Sentinelles* GP surveillance network in France [25]. In one area in France, Franche-Comté [36], and one area in Germany, Bavaria (Lyme Disease Incidence-Study: LYDI-Sentinel) [35], cases were reported prospectively by a network of both GPs and medical specialists. Two reports provided results from mandatory notification systems in Germany at a regional level: for physicians and laboratories in six eastern states [26] and for physicians in Bavaria [35]. The duration of the different study periods ranged between one and four years. The total number of confirmed LB cases included in the studies ranged from 282 to 18,894 patients. Multiple EM, although a disseminated manifestation, was included in the group of EM patients in all studies.

Quality assessment

As requested in the inclusion criteria, case definitions used in the studies included were in line with the European case definitions [10], with some small modifications made (Appendix C, Table C2). Compliance of the reported cases with the case definitions was checked in all studies. This was done for all cases, except in the Dutch study which validated part of their disseminated LB cases only (the results were extrapolated to all disseminated cases). As an additional validation, Hofhuis et al. screened cases of GPs reporting the 10% highest incidence and excluded them if clearly deviating answers or unsatisfactory internal consistency was found [14]. The French study by Vandenesch et al. (2014), the LYDI-Sentinel study in Bavaria [35] and the study in Franche-Comté [36] examined the cases and contacted physicians when necessary (e.g., missing or inconsistent data). The Bavarian mandatory reporting screened and corrected cases, at least for the first year, to increase data exhaustiveness. Subsequently, measures (e.g., revision of reporting form) were undertaken to allow future data collection without manual data correction [37]. With exception of the Dutch study, a more detailed description on the age and gender of the included cases and a seasonality description were available in all studies. A bimodal age distribution (peak in 5-10 year-old and in 45-70 year-old) was observed in the studies for which these data were available. A

slightly higher percentage of females affected and a clear seasonality was reported by all the studies [14,25,26,35,36]. None of the studies were excluded because of poor quality.

Data extraction

The primary data of included studies are provided in Table 1.

Table 1. Absolute numbers of clinical manifestations of Lyme borreliosis in the studies included in the systematic review and meta-analysis

Source	EM[a]	LA	LNB	OTH
Hofhuis et al., 2015 *(+ personal communication)*	11,442	234	198	156
Vandenesch et al., 2014	309	10	6	7[b]
Wilking et al., 2014	18,016	367	630[c]	N/A
Heinzinger et al., 2017; Mandatory	15,797	373	302[c]	N/A
Heinzinger et al., 2017; LYDI	276	9	1[c]	N/A
Serre et al., 2017	394	4	20	5

EM: erythema migrans; LA: Lyme arthritis; LNB: Lyme neuroborreliosis; OTH: other manifestations; N/A: not available

[a] Multiple erythema migrans was included in the EM group.

[b] This number includes all other manifestations except ocular manifestations.

[c] Only acute LNB was included in the study.

Out of the 168 other LB manifestations in the three studies with available data, there were 53 cases (32%) with borrelial lymphocytoma, 95 cases (57%) with ACA, 11 (7%) with Lyme carditis and 9 (5%) with ocular manifestations [14,25,36].

Meta-analysis

The pooled estimates of the ratios of LNB, LA and other manifestations to the number of EM manifestations are summarized in Table 2.

Table 2. Ratios of LA, EM and other LB manifestations to EM manifestations

	Ratio	95% confidence interval
LNB/EM	0.024	0.016–0.037
LA/EM	0.022	0.020–0.024
OTH/EM[a]	0.014	0.012–0.016

EM: erythema migrans; LNB: Lyme neuroborreliosis; LA: Lyme arthritis; OTH: other manifestations
[a] Studies without results for other manifestations (N=3) excluded from analysis.

The results of a scenario analysis excluding the studies based on mandatory notification are presented in Appendix D, Table D1. No important differences were observed.

Data synthesis

Table 3 presents the estimated annual incidences of the main clinical manifestations of LB in Belgium based on the annual incidence of EM (2015-2017) and the ratios of the other manifestations to EM, estimated in the meta-analysis.

Table 3. Estimated annual incidences and absolute numbers of cases of EM, LA, LNB and other LB manifestations in Belgium, period 2015-2017

	Annual incidence per 100,000	95% UI	Absolute number cases[a] + 95% UI
EM	97.6	82.0–113.0	11,022 (9,267–12,768)
LNB	2.4	1.5–3.7	270 (164–418)
LA	2.1	1.7–2.6	240 (195–289)
OTH	1.4	1.1–1.7	154 (120–192)
Total LB[b]	103.5	86.9–119.8	11,685 (9,821–13,537)

EM: erythema migrans; LNB: Lyme neuroborreliosis; LA: Lyme arthritis; OTH: other manifestations; UI: uncertainty interval
[a] Based on mid-year population Belgium, 2016 (Public Health and Surveillance, 2017).
[b] Total clinical LB manifestations.

Discussion

Based on the results of the SGP network, the annual incidence of EM in Belgium in 2015-2017 (97.6 cases/100,000 inhabitants (95% UI 82.0–113.0)) is comparable to the incidence in 2008-2009 (93.9/100,000 (95% UI 85.0-102.9)) (as presented by Vanthomme et al. (2012), but recalculated based on current methodology [7]). Other data sources also did not indicate a significant increase of LB in the Belgian population over the past decade [8,9,38]. Although both GP-related and patient-related factors may contribute to differences in notification of EM by the SGP network in the different provinces, the observed higher incidences in Limburg, Antwerp and Luxemburg are consistent with results of laboratory surveillance of positive serology tests and reporting of tick bites in Belgium through an online citizen-based platform [8,9,39]. Important regional differences also exist in other European countries [14,25,26].

Only six studies were found that met the inclusion criteria of the systematic review. As expected, EM was the most prevalent manifestation in all these studies. According to our meta-analysis, LNB and LA occur in similar proportions (ratio to EM of 0.024 and 0.022 respectively) and other LB manifestations are relatively rare (ratio of 0.014, joint in one group). The proportion of EM (93-97%) reported in the six studies included in the systematic review and meta-analysis was higher in comparison with other, often older, studies from European countries, but these data often had their own methodological limitations [4,40–47]. The only available data on the frequency of the different manifestations for Belgium are reported by a study by Bigaignon et al. in 1989 [48]. In this study, only seropositive patients were included, explaining the lower proportion of EM (63%), as laboratory tests may yield negative results in this early disease stage. Likewise, a lower proportion of EM (range 60% - 91%) is seen in other studies or surveillance reports based on laboratory criteria only and/or in hospital-based studies [28,33,49–51]. Furthermore, an increased awareness on LB in the past decades might have caused a shift towards diagnosis and treatment in an earlier

disease stage, avoiding more disseminated and late manifestations [52]. Therefore, only data from the most recent ten years were included in our review. Also, the meta-analysis focused on neighboring countries only, as we expect them to be more comparable with regard to geographical and climatic characteristics, to the prevalence of the *Borrelia* species in ticks [16] and to changes in awareness on LB.

The risk of bias in the results of the systematic review and meta-analysis is dependent on the primary data included as these have their individual risk of bias. This mainly consists of possible disproportionate over- or underestimation of one of the clinical manifestations compared to the others. This is influenced by the study design or surveillance method of the studies included, but also by the healthcare system that is in place in the concerned country [53]. Ideally, a random sample representative of all physicians diagnosing manifestations of LB in a country (i.e., general practitioners, infectious disease specialists, dermatologists, neurologists, rheumatologists, pediatricians and cardiologists) would report the number of cases, with a 100% compliance and sufficient clinical and laboratory details to check accordance with the case definitions. In reality, this is not available for any country, nor feasible. The studies from France and the Netherlands are both based on GP reporting only and might underestimate the proportion of manifestations other than EM as these are more severe and difficult to diagnose, and therefore more often seen by medical specialists. Nevertheless, in both countries patients are obliged to visit a GP before referral to a specialist and it is expected that the participating GPs report both on cases diagnosed by themselves as on those diagnosed by the specialists [14,25,54]. In practice this might not always be the case and there might still be an overestimation of the proportion of EM [25]. In the studies in Franche-Comté and Bavaria (LYDI-Sentinel) both GPs and specialists are reporting cases. Nevertheless, the ratio of other LB manifestations to EM in the study in Franche-Comté is comparable to the national GP study in France [25,36]; in the LYDI-Sentinel study (Bavaria) the ratio is even lower compared to the other studies. The surveillance based on mandatory notification (two studies in Germany) has

the advantage that it is obligatory for all types of physicians (and laboratories, for the study by Wilking et al. (2014)), but in practice it could be prone to underreporting [53,55–57]. It is true that LB incidence in Germany has previously been estimated to be higher based on a prospective, population-based study in the Würzburg region in Central-Germany in 1999 [43]. However, since only proportions of different manifestations and not the incidences are included in our analysis, and because of the important advantage of participation of all types of physicians, these studies were included in the meta-analysis. Note that underreporting may be higher for EM than for the other manifestations as it is less severe and easy to treat [26,57,58]. Another limitation from the study by Wilking et al. (2014) is that in one state, mandatory notification only applies to the laboratories through which EM is less often diagnosed and thereby may be underestimated. Furthermore, it could be possible that some cases are reported twice, by a physician and laboratory. Despite the heterogeneity between the studies included in the review (different study designs) they resulted in comparable estimations of the proportion of the clinical manifestations. In addition, the possible impact of exclusion of the mandatory notification data from our meta-analysis was checked through an alternative scenario; the final ratios estimated were very similar to the results presented (Appendix D).

Our study allowed estimating for the first time, the annual incidences of other Lyme borreliosis manifestations than EM, based on routine EM surveillance and on information from neighboring countries. Nevertheless, there are some limitations to this study. Some concern the estimation of the EM incidence itself. The Belgian SGP network is based on a voluntary participation of GPs who have to declare cases actively. Underreporting is possible, especially in districts less affected by LB due to lower awareness [12]. In addition, as an EM disappears after some time, and as patients may not always be aware of the necessity of treatment, cases might not always seek medical care (= under-ascertainment). This is a common limitation with all the studies included in the meta-analysis. Both underreporting and under-ascertainment lead to

underestimation, but the exact magnitude of this problem is unknown. On the other hand, erroneously diagnosing other skin rashes as an EM could lead to overestimation [11,12,59]. To improve data quality, the SGPs receive a clinical case definition and instructions on a yearly basis. However, no further validation of the reported cases is carried out.

Other limitations are related to the systematic review and meta-analysis. Firstly, as inclusion criteria were broad, differences in study and surveillance methodologies between included data existed. A random effects meta-analysis was used to explicitly take into account these differences in the meta-analysis. Secondly, in three out of six included studies, the patient sample size was rather small [25,35,36]. Since LB manifestations other than EM, LNB and LA (ACA, Borrelial lymphocytoma, Lyme carditis and ocular manifestations) were not included in the German studies, less data could be included in the analysis of their ratio to EM. Furthermore, in one of the remaining studies ocular manifestations were not included [25]. The German studies also only considered early LNB and not late LNB cases [26,35]. As the latter is extremely rare in Europe (< 2-5% of all LNB), this was not adjusted for in the meta-analysis [60,61]. In general, we believe that an underestimation of the proportion of LB manifestations other than EM is possible as the included studies based on GP reporting might be prone to underestimation of these manifestations as patients may visit a specialist directly. Furthermore, the decision to include only studies based on confirmed LB cases is also likely to have caused an underestimation of these LB manifestations other than EM: as more specific data are required for a confirmed diagnosis, including clinical and laboratory data (serum ELISA and Western blot, cerebrospinal fluid analysis, intrathecal antibody production, pleocytosis,…) [10], chances are higher that these manifestations are classified as probable or possible due to missing information and thereby not included in our analysis.

Finally, the incidence estimates are based on reported cases only. The true incidences may be higher due to under-ascertainment (not all patients seek healthcare, mainly for EM) and underreporting (for all manifestations). Additional studies may allow assessing this underestimation and better estimating the exact number of the different LB manifestations, including probable cases.

Conclusion

An estimation of the incidence of the different clinical manifestations of Lyme borreliosis is useful to follow-up disease trends and necessary to estimate the health burden and costs of the disease in a country. However, collecting representative data on all the different manifestations is very complex and time-consuming. This study used routine surveillance data of EM and data available from other (neighboring) countries to estimate the incidence of other, less frequent manifestations. Future studies to validate these estimates would be of great value.

Ethics committee approval

The Ethical Committees of the Scientific Society of Flemish GPs and the Catholic University of Louvain (UCL) approved the Belgian network of SGP in its entirety.

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We are very grateful to the sentinel GPs involved in the data collection. We also acknowledge all contacted authors of the articles included in the systematic review and their colleagues, for additional information regarding their studies, and thank Agnetha Hofhuis for the personal communication of more detailed data.

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Appendices

Table of Contents

Appendix A

Table A1. PRISMA 2009 Checklist

Section/topic	#	Checklist item	Reported on page
Title			
Title	1	Identify the report as a systematic review, meta-analysis, or both.	51
Abstract			
Structured summary	2	Provide a structured summary including, as applicable: background; objectives; data sources; study eligibility criteria, participants, and interventions; study appraisal and synthesis methods; results; limitations; conclusions and implications of key findings; systematic review registration number.	52
Introduction			
Rationale	3	Describe the rationale for the review in the context of what is already known.	53-54
Objectives	4	Provide an explicit statement of questions being addressed with reference to participants, interventions, comparisons, outcomes, and study design (PICOS).	54
Methods			
Protocol and registration	5	Indicate if a review protocol exists, if and where it can be accessed (e.g., Web address), and, if available, provide registration information including registration number.	NA
Eligibility criteria	6	Specify study characteristics (e.g., PICOS, length of follow-up) and report characteristics (e.g., years considered, language, publication status) used as criteria for eligibility, giving rationale.	55-56
Information sources	7	Describe all information sources (e.g., databases with dates of coverage, contact with study authors to identify additional studies) in the search and date last searched.	55-56
Search	8	Present full electronic search strategy for at least one database, including any limits used, such that it could be repeated.	Appendix B
Study selection	9	State the process for selecting studies (i.e., screening, eligibility, included in systematic review, and, if applicable, included in the meta-analysis).	56
Data collection process	10	Describe method of data extraction from reports (e.g., piloted forms, independently, in duplicate) and any processes for obtaining and confirming data from investigators.	57

Section/topic	#	Checklist item	Reported on page
Data items	11	List and define all variables for which data were sought (e.g., PICOS, funding sources) and any assumptions and simplifications made.	56-57
Risk of bias in individual studies	12	Describe methods used for assessing risk of bias of individual studies (including specification of whether this was done at the study or outcome level), and how this information is to be used in any data synthesis.	56
Summary measures	13	State the principal summary measures (e.g., risk ratio, difference in means).	56
Synthesis of results	14	Describe the methods of handling data and combining results of studies, if done, including measures of consistency (e.g., I^2) for each meta-analysis.	56
Risk of bias across studies	15	Specify any assessment of risk of bias that may affect the cumulative evidence (e.g., publication bias, selective reporting within studies).	Discussion p. 65-69
Additional analyses	16	Describe methods of additional analyses (e.g., sensitivity or subgroup analyses, meta-regression), if done, indicating which were pre-specified.	57
Results			
Study selection	17	Give numbers of studies screened, assessed for eligibility, and included in the review, with reasons for exclusions at each stage, ideally with a flow diagram.	60 & Figure 4 p. 61
Study characteristics	18	For each study, present characteristics for which data were extracted (e.g., study size, PICOS, follow-up period) and provide the citations.	61-62 & Appendix C, Table C1
Risk of bias within studies	19	Present data on risk of bias of each study and, if available, any outcome level assessment (see item 12).	62-63 & Discussion
Results of individual studies	20	For all outcomes considered (benefits or harms), present, for each study: (a) simple summary data for each intervention group (b) effect estimates and confidence intervals, ideally with a forest plot.	Table 1
Synthesis of results	21	Present results of each meta-analysis done, including confidence intervals and measures of consistency.	Table 2
Risk of bias across studies	22	Present results of any assessment of risk of bias across studies (see Item 15).	Discussion p. 65-69
Additional analysis	23	Give results of additional analyses, if done (e.g., sensitivity or subgroup analyses, meta-regression [see Item 16]).	Appendix D

Section/topic	#	Checklist item	Reported on page
Discussion			
Summary of evidence	24	Summarize the main findings including the strength of evidence for each main outcome; consider their relevance to key groups (e.g., healthcare providers, users, and policy makers).	65-66
Limitations	25	Discuss limitations at study and outcome level (e.g., risk of bias), and at review-level (e.g., incomplete retrieval of identified research, reporting bias).	66-69
Conclusions	26	Provide a general interpretation of the results in the context of other evidence, and implications for future research.	65, 69
Funding			
Funding	27	Describe sources of funding for the systematic review and other support (e.g., supply of data); role of funders for the systematic review.	Published article

From: Moher D, Liberati A, Tetzlaff J, Altman DG, The PRISMA Group (2009). Preferred Reporting Items for Systematic Reviews and Meta-Analyses: The PRISMA Statement. PLoS Med 6(7): e1000097. doi:10.1371/journal.pmed1000097

For more information, visit: **www.prisma-statement.org**.

Appendix B

Table B1. Database search strategy

	In PubMed	In Embase
#1:	lyme disease[a]	'lyme disease'/exp OR 'lyme disease' [b]
#2:	epidemiology	'epidemiology'/exp OR 'epidemiology'
#3:	incidence	'incidence'/exp OR 'incidence'
#4:	prevalence	'prevalence'/exp OR 'prevalence'
#5:	clinical manifestation*	'clinical manifestation*'
#6:	belgium	'belgium'/exp OR 'belgium'
#7:	france	'france'/exp OR 'france'
#8:	netherlands	'netherlands'/exp OR 'netherlands'
#9:	germany	'germany'/exp OR 'germany'
#10:	luxembourg	'luxembourg'/exp OR 'luxembourg'
#11:	united kingdom	'united kingdom'/exp OR 'united kingdom'
#12:	"animals"[MeSH Terms]	'animal'/exp
#13:	"humans"[MeSH Terms]	'human'/exp
#14:	#2 OR #3 OR #4 OR #5	#2 OR #3 OR #4 OR #5
#15:	#6 OR #7 OR #8 OR #9 OR #10 OR #11	#6 OR #7 OR #8 OR #9 OR #10 OR #11
#16:	#12 NOT (#12 AND #13)	#12 NOT (#12 AND #13)
#17:	#1 AND #14 AND #15	#1 AND #14 AND #15
#18:	#17 NOT #16	#17 NOT #16
#19:	#18 **Filters:** Publication date from 2008/02/01 to 2018/01/31; Dutch; English; French; German	#18 AND [1-2-2008]/sd NOT [31-1-2018]/sd AND ([dutch]/lim OR [english]/lim OR [french]/lim OR [german]/lim)

[a] As no restrictions are specified, PubMed will automatically search for 'Lyme disease' [MeSH] (thereby including synonym entry terms such as 'Lyme Borreliosis') as well as search for 'Lyme' And 'disease' or 'Lyme disease' in [All Fields]. Subsequent search terms (epidemiology, incidence, etc.) are also automatically converted to subheadings or MeSH terms and searched for as free-text in [All Fields].

[b] By specifying /exp, Embase will include 'Lyme disease' as an Emtree term in the search. Lyme disease is the preferred Emtree term for 'Lyme Borreliosis' and other related synonyms. Subsequent search terms are also converted to Emtree terms.

Table B2. Grey literature (national public health institute websites)

Country	Website public health institute
Belgium	https://www.sciensano.be/nl/gezondheidsonderwerpen/ziekte-van-lyme/cijfers
France	http://invs.santepubliquefrance.fr/Dossiers-thematiques/Maladies-infectieuses/Maladies-a-transmission-vectorielle/Borreliose-de-lyme/Publications
The Netherlands	https://www.rivm.nl/Onderwerpen/Z/Ziekte_van_Lyme#scientific_article
Germany	https://www.rki.de/DE/Content/InfAZ/B/Borreliose/Borreliose.html?cms_box=1&cms_current=Borreliose+%28Lyme-Borreliose%29&cms_lv2=2398670
Luxembourg	http://www.sante.public.lu/fr/maladies/zone-corps/sang/maladie-lyme/index.html
United Kingdom	https://www.gov.uk/government/collections/lyme-disease-guidance-data-and-analysis

Appendix C

Table C1. Characteristics of included studies

Reference	Country, region; Study period	Study methods: Design - Reporting entity – Coverage - Reporting type	Manifestations included in the study	Data collection	Incidence (/100,000 inhabitants)
Hofhuis et al., 2015 [1]	The Netherlands; 2009-2010 [a]	• Cross-sectional retrospective survey • General practitioners[b] • Comprehensive (estimation based on 39% of GPs) • Voluntary	• EM • LNB • LA • BL • ACA • Lyme carditis • Ocular manifestations	Questionnaires by post: 2 step: retrospective questionnaire sent to all GPs + validation questionnaire in second step for disseminated cases	EM: 131.5 (95% CI 127.1-136) Disseminated LB: 7.7/100,000 (95% CI 7.2-8.2)
Vandenesch et al., 2014 [2]	France; 2009-2012	• Prospective surveillance by sentinel reporting • General practitioners • Sentinel • Voluntary	• EM • LNB • LA • BL • ACA • Lyme carditis	Weekly notification to electronic system. Standardized questionnaire for each notified case	42 (95% CI 37-48)
Wilking and Stark, 2014 [3]	Germany, 6 eastern states; 2009-2012	• Surveillance by mandatory notification system • Physicians (5/6 states), Laboratories (4/6 states) • Comprehensive • Mandatory	• EM • Acute LNB • LA	Electronic reporting to the federal state level health authority and from there to the Robert Koch Institute	Varied between 34.9 (in 2009) and 19.54 (in 2012)
Heinzinger et al., 2017; Mandatory [4]	Germany, Bavaria; 2013-2016	• Surveillance by mandatory notification system • Physicians • Comprehensive • Mandatory	• EM • Acute LNB • LA	Registration form Transmission method not specified	34

Reference	**Country, region; Study period**	**Study methods:** Design - Reporting entity – Coverage - Reporting type	**Manifestations included in the study**	**Data collection**	**Incidence (/100,000 inhabitants)**
Heinzinger et al., 2017; LYDI [4]	Germany, Bavaria; 2013-2016	• Prospective surveillance through sentinel reporting • General practitioners, dermatologists, neurologists, rheumatologists • Sentinel • Voluntary	• EM • Acute LNB • LA	Questionnaire per patient	/
Serre et al., 2017 [5]	France, Franche-Comté, 1 April 2010 – 31 March 2012	• Prospective study • GPs, dermatologists, pediatrics, rheumatologists, neurologists, cardiologists, infectious disease specialists, internal medicines • Comprehensive (estimation based on 15% of physicians) • Voluntary	• EM • LNB • LA • BL • ACA • Lyme carditis • Ocular manifestations	Questionnaire for each patient, sent by fax/post, from august 2010 also online.	85 (95% CI 77-92)

EM: erythema migrans; LNB: Lyme neuroborreliosis; LA: Lyme arthritis; BL: Borrelial lymphocytoma; ACA: acrodermatitis chronica atrophicans; LB: Lyme borreliosis; CI: confidence interval

[a] 2009-2010 for disseminated manifestations, 2010 for EM.

[b] Exclusion specialists' data.

Table C2. Modifications[a] of study case definitions in comparison with the European case definitions (Stanek et al. 2011)

Reference	**Hofhuis et al., 2015** [1]	**Vandenesch et al., 2014** [2]	**Wilking et al., 2014** [3]	**Heinzinger et al., 2017; Mandatory**	**Heinzinger et al., 2017; LYDI**	**Serre et al. 2017** [5]
Definitions referred to in article	**Stanek et al. 2011** [6]	**Stanek et al. 2011** [6]	**RKI (2009)** [7]	**RKI (2009)** [7]	**Definitions described in earlier data**	**Stanek et al. 2011** [6]
EM (+ MEM)	No modification	No modification	EM diameter ≥ 5 cm	EM diameter ≥ 5 cm	EM diameter ≥ 5 cm	No modification
Lyme Arthritis[b]	No modification	No modification	In line with Stanek et al. 2011	In line with Stanek et al. 2011	In line with Stanek et al. 2011	No modification
LNB[b]	No modification	Meningoradiculitis or unilateral facial paralysis cases were validated even if CSF fluid analysis had not been done, if clinically very suggestive (agreement three authors) in patients who reported a history of EM less than two months before the onset of neurological manifestations.	**Only acute LNB** - Pleocytosis not necessary for cerebral nerve paralysis - IgG antibody detection sufficient for cranial neuritis and persons ≤18 years	**Only acute LNB** - Pleocytosis not necessary for cerebral nerve paralysis - IgG antibody detection sufficient for cranial neuritis and persons ≤18 years	**Only acute LNB** - CSF pleocytosis may be absent in very short illness duration	- If intrathecal antibody production is not checked, lymphocytosis and positive serology in CSF are sufficient. - Facial paralysis in children < 15 year old with positive serology, without CSF puncture is sufficient
BL (rare)[b]	No modification	No modification	Not reported	Not reported	Not reported	No modification
ACA[b]	No modification	No modification	Not reported	Not reported	Not reported	No modification
Carditis (rare)[b]	No modification	No modification	Not reported	Not reported	Not reported	No modification
Ocular (rare)[b]	No modification	No modification	Not reported	Not reported	Not reported	No modification

(M)EM: (Multiple) erythema migrans; LNB: Lyme neuroborreliosis; BL: Borrelial lymphocytoma; ACA: acrodermatitis chronica atrophicans

[a] Only modifications with a possible impact on the number of cases included in the study are specified.

[b] In all countries, serology follows a two-step approach: positive screening (ELISA) + confirmation (Western blot/Line assay).

Appendix D: Scenario analysis

The impact of exclusion of surveillance data based on mandatory notification from the systematic review, meta-analysis and data synthesis was explored in an additional scenario analysis:

Table D1. Absolute numbers of clinical manifestations of Lyme borreliosis in the studies included in the alternative scenario

Source	EM[a]	LA	LNB	OTH
Hofhuis et al., 2015 [1] *(+ personal communication)*	11,442	234	198	156
Vandenesch et al., 2014 [2]	309	10	6	7[b]
Heinzinger et al., 2017; LYDI [4]	276	9	1[c]	N/A
Serre et al., 2017 [5]	394	4	20	5

EM: erythema migrans; LA: Lyme arthritis; LNB: Lyme neuroborreliosis; OTH: other manifestations; N/A: not available

[a] Multiple erythema migrans was included in the EM group.

[b] This number includes all other manifestations except ocular manifestations.

[c] Only acute LNB was included in the study.

Table D2. Ratios of LA, EM and other LB manifestations to EM manifestations

	Ratio	95% confidence interval
LNB/EM	0.023	0.010–0.046
LA/EM	0.023	0.016–0.031
OTH/EM[a]	0.014	0.012–0.016

EM: erythema migrans; LNB: Lyme neuroborreliosis; LA: Lyme arthritis; OTH: other manifestations

[a] Studies without results for other manifestations excluded from analysis.

Table D3. Estimated annual incidence and absolute numbers of cases of EM, LA, LNB and other LB manifestations in Belgium, period 2015-2017

	Annual incidence per 100,000	**95% UI**	**Absolute number cases[a] + 95% UI**
EM	97.6	82.0–113.0	11,022 (9,267–12,768)
LNB	2.2	0.9–4.5	249 (103–510)
LA	2.2	1.5–3.2	253 (171–361)
OTH	1.4	1.1–1.7	154 (120–192)
Total LB[b]	103.4	86.8–119.9	11,678 (9,802–13,542)

EM: erythema migrans; LNB: Lyme neuroborreliosis; LA: Lyme arthritis; OTH: other manifestations; UI: uncertainty interval

[a] Based on mid-year population Belgium, 2016 [9].

[b] Total clinical LB manifestations.

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Chapter 5

Non-specific symptoms and post-treatment Lyme disease syndrome in patients with Lyme borreliosis: a prospective cohort study in Belgium, 2016-2020

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Submitted

Abstract

Background: Patients with Lyme borreliosis (LB) may report persisting non-specific symptoms such as fatigue, widespread musculoskeletal pain or cognitive difficulties. When present for more than 6 months and causing a reduction in daily activities, this is often referred to as post-treatment Lyme disease syndrome (PTLDS). This study aimed to compare the occurrence of symptoms between LB patients and controls and to estimate the proportion of LB patients developing PTLDS and to identify risk factors. **Methods:** A prospective cohort study was set up including three subpopulations: patients with an erythema migrans (EM) (i), disseminated/late LB (ii) and a non-LB control group (iii). At 6 and 12 months follow-up, the study compared the occurrence of several symptoms, including six symptoms used to define PTLDS and impact on daily activities, between LB patients and controls. Ultimately, we estimated the proportion of LB patients developing PTLDS as defined by the Infectious Disease Society of America, including a time frame for symptoms to be present. **Results:** Although the risk of presenting PTLDS-related symptoms was significantly higher in EM patients (n=120) compared to controls (n=128) at 6 months follow-up, the risk of presenting at least one of these symptoms combined with impact on daily activities was not significantly higher in EM patients, at either 6 or 12 months follow-up. A significant association was found between disseminated/late LB (n=15) and the occurrence of any PTLDS-symptom with an impact on daily activities at both time points. The proportion of patients with PTLDS was estimated at 5.9% (95% CI 2.7-12.9) in EM patients and 20.9% (95% CI 6.8-64.4) in disseminated/late LB (RR = 3.53, 95% CI 0.98-12.68, p=0.053). No significant risk factors were identified, which may be explained by small sample sizes. **Conclusions:** In our study, PTLDS was present in both LB cohorts, yet with a higher percentage in disseminated/late LB patients. Additional research is needed into risk factors for and causes of this syndrome and further development and validation of standardized methods to assess the PTLDS case definition, easily applicable in practice, is of great importance.

Introduction

Lyme borreliosis (LB) is an important tick-borne disease caused by spirochetes of the *Borrelia burgdorferi* sensu lato (s.l.) complex. As the spirochetes can affect different sites of the body, it can manifest with a broad variety of clinical symptoms, yet asymptomatic infections also occur [1,2]. The most common manifestation is an erythema migrans (EM), an early-localized infection, possibly accompanied with flu-like symptoms. When left untreated, early disseminated LB or late LB may develop, amongst others, as multiple erythema migrans (MEM), Lyme neuroborreliosis (LNB), Lyme arthritis (LA), Lyme carditis or acrodermatitis chronica atrophicans (ACA) [1–3]. In addition to these objectively identifiable manifestations, it has been reported that a subset of patients continues to experience non-specific symptoms even despite adequate antibiotic treatment. Frequencies of these symptoms reported in literature vary widely, between 5 and 36% in EM patients, with the highest frequency in a study on EM patients also presenting with systemic symptoms (e.g., viral-like symptoms) [4–11], and up to about 50% in some studies in LNB patients [12–15]. However, substantial heterogeneity exists in the case definitions used, study designs and population under study. In order to better delineate this group of patients and to improve research on the occurrence and pathogenesis of these symptoms, a case definition for post-Lyme disease syndrome (PLDS), now mostly referred to as post-treatment Lyme disease syndrome (PTLDS), was proposed by the Infectious Disease Society of America (IDSA) in 2006 [16]. This definition includes the onset of fatigue, widespread musculoskeletal pain or complaints of cognitive difficulties within 6 months of a proven *B. burgdorferi* infection, symptoms persisting for ≥ 6 months (continuous or relapsing) after appropriate antibiotic therapy which led to resolution or stabilization of objective manifestations, and the symptoms being of such severity that, when present, they result in substantial reduction in previous levels of occupational, educational, social, or personal activities. In addition, several exclusion criteria were proposed [16]. As the symptoms of PTLDS are also very prevalent in the general population, controversy still

exists on the frequency, cause and treatment of the syndrome. For the latter, several randomized controlled treatment trials showed insufficient evidence of the benefit of prolonged antibiotic treatment in patients with persisting symptoms [17–21].

The aim of this study was twofold: first, to compare non-specific symptoms as such and symptoms impacting daily activities occurring after LB between LB patients and a non-LB control group at 6 and 12 months follow-up; second, to estimate the proportion of PTLDS in two groups of LB patients - patients with an erythema migrans (i) and disseminated or late LB (ii) - according to the full IDSA case definition including the time frame proposed, and to identify possible risk factors. To allow comparison with previously reported results, different steps were followed to present and discuss the study results.

Methods

Study design & participant selection

This study was part of a larger project, the HUMTICK study, for which the initial study protocol has been described previously [22]. HUMTICK is a prospective cohort study in which patients diagnosed with an EM (i) or disseminated/late LB manifestations (ii) were followed up for 6 to 24 months after diagnosis and treatment, and a non-LB control group (iii) was concurrently followed up during 6 to 12 months. Participants, aged 18 years or older, were included between June 2016 and December 2019. EM patients were recruited by a network of 200 general practitioners and patients with disseminated/late LB manifestations by medical specialists in 8 hospitals, all located in areas in Belgium that are highly endemic for tick bites and LB. Case definitions for inclusion were published in the study protocol [22] and can be found in Appendix A [23, 24]. In these, EM is clinically diagnosed by the GP whereas other manifestations are based on both clinical and laboratory criteria. Multiple EM, although a disseminated manifestation, was included in the group of EM patients (i). If the EM diameter was < 5

cm and no tick bite was recalled or the delay in EM appearance was less than two days (if known), the diagnosis was classified as possible EM and the patient was not included in the baseline analysis. LNB patients included as a case by the hospital based on high suspicion, but without a cerebrospinal fluid analysis performed at diagnosis were similarly not included in the baseline analysis. Participants of the non-LB control group were selected by patients among persons in their environment with the same gender and age ± 5 years and no prior LB diagnosis (self-reported, no serology performed). As not all patients were able to provide a control person, additional controls were recruited by the participating controls (with the same requirements) and through online invitations of persons in gender and age categories lacking controls at the end. Pregnant patients were excluded.

Evaluation of participants

LB patients were asked to fill in a questionnaire at diagnosis (T0), 1 and 3 months later (T1 & T3), and at 6, possibly 12 and 24 months after treatment completion (T6, T12 & T24), depending on the moment of enrollment and time left until the end of the study period. In line with the IDSA case definition for PTLDS, the questionnaires assessed the presence of subjective symptoms, as well as the impact on occupational, educational, social or personal activities, hereafter referred to as daily activities [16]. Patients' health before and during the onset of their LB was assessed in the first questionnaire, post-treatment health in the follow-up questionnaires from 3 months onwards. The same questions were answered by the control group at T0 (inclusion) and T6, possibly at T12 (depending on enrollment moment).

Symptoms

Six symptoms, namely, muscle pain (> 1 part of the body), joint pain (> 1 part of the body), fatigue, memory problems (forgetfulness), difficulties concentrating and problems finding words – were used throughout the study to assess the symptoms included in the IDSA definition for PTLDS, henceforth referred to as PTLDS-related

symptoms. At follow-up, five additional non-specific symptoms – headache, sensory disorders (e.g., tingling), night sweats (with waking up), excessive sleep and troubles falling asleep – were added to the list, as these have been suggested in literature to be related to PTLDS [25]. Presence of swollen joints was added as an objective symptom, leading to a 12-item symptom list. For each symptom, patients reported whether or not it had regularly been present (pre-Lyme or since the previous questionnaire), the cause if known, and for each symptom present, if it was more severe, equally severe or less severe at follow-up in comparison with their pre-Lyme general health (or pre-participation health for the control group). Furthermore, three standardized questionnaires, assessing the severity of the PTLDS-related symptoms were added:

1. SF-36 bodily pain (SF-36 BP) subscale to assess widespread musculoskeletal pain during the past 4 weeks [26,27]
2. SF-36 vitality (SF-36 VT) subscale to assess fatigue during the past 4 weeks [26,27]
3. Cognitive Failure Questionnaire (CFQ) to assess cognitive difficulties during the past months [28]

At T0, to assess pre-Lyme health, the periods covered by the questionnaires were adapted to 4 weeks (SF-36) or the months (CFQ) before the tick bite or the start of LB complaints.

Impact on daily activities

At follow-up, a modified version of the global activity limitation indicator (GALI, a three-level multiple choice question) was used to assess the impact of the 12 symptoms on the patient's life, i.e., patients were asked whether they were limited in their daily activities (e.g., work, school, hobbies, or others) due to these symptom(s) [29,30]. Furthermore, a part of the standardized questionnaire EQ-5D-5L, a question on having problems with usual activities in general (e.g., work, study, housework, family or leisure activities), was added, assessing the past 4 weeks [31,32].

Outcome definitions

Comparison of patients vs. controls at follow-up

Following a step-wise approach, different outcomes were defined. In a comparison between LB patients and the non-LB control group at 6 and 12 months follow-up, first, the new occurrence or worsening of each symptom of the 12-item symptom list was compared as such. Second, the comparison of symptoms was narrowed down to the occurrence of at least one of the six PTLDS-related symptoms and an impact on daily activities was added as a criterion. Details on the exact definitions used are shown in Table 1.

Table 1. Outcome definitions in the comparison of patients vs. controls at 6 and 12 months follow-up, HUMTICK study, Belgium, 2016-2020

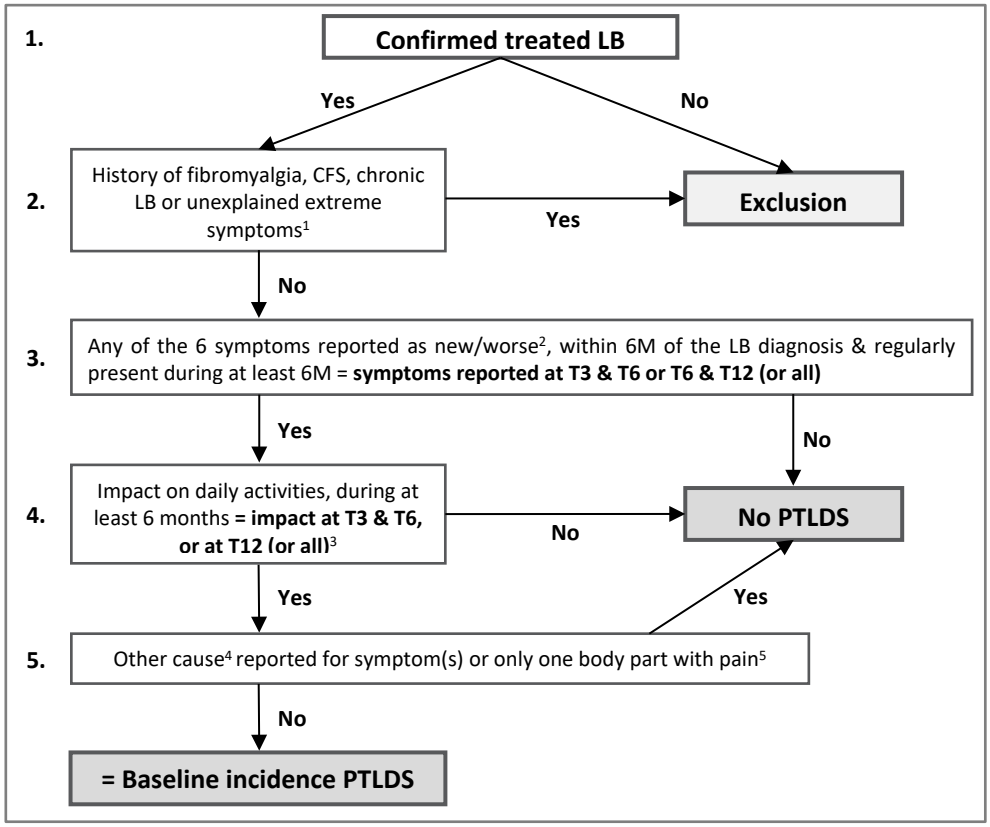
1.	**Occurrence of new or worsened non-specific symptoms (12 symptoms):**
	New or worsened symptom[1] =
	- regularly present since the previous questionnaire AND - "more severe" compared to pre-Lyme or pre-participation health AND - worse standardized questionnaire score for symptom present (only for the 6 PTLDS-related symptoms based on SF-36 BP, SF-36 VT or CFQ)
2.	**Occurrence of any of the 6 PTLDS-related symptoms:**
	At least one of the 6 PTLDS-related symptoms new or worsened
	- Defined as above[1]
	+ impact daily activities =
	- limited or strongly limited on the modified GALI AND - at least slight problems with daily activities on the question of the EQ-5D-5L (= level 2-5)

[1] Note that these symptoms can have other possible causes as no exclusion was performed at this stage.

PTLDS in LB patients

Third, to estimate the proportion of patients developing PTLDS after erythema migrans (i) and after disseminated/late LB manifestations (ii), additional criteria of the IDSA case definition were applied to the two cohorts as shown in Figure 1. In order to include the time frame (symptom start < 6 months, duration ≥ 6 months), different time points were combined in the analysis. In the baseline scenario, the definitions as described in Table 1 were used to define new or worsened symptoms and impact on daily activities. In addition to the baseline scenario, the incidence of PTLDS was estimated following four alternative scenarios using different inclusion or outcome criteria (Appendices).

Figure 1. Decision tree for defining post-treatment Lyme disease cases following IDSA definition [16]



LB: Lyme borreliosis, CFS: chronic fatigue syndrome, 6M: 6 months, T3: 3 months after diagnosis, T6 and T12: 6 and 12 months after treatment

[1] unexplained or undiagnosed extreme fatigue, widespread musculoskeletal pain or cognitive difficulties.
[2] Widespread musculoskeletal pain expressed as muscle pain (1) or joint pain (2) at more than one place of the body, fatigue (3), or cognitive difficulties expressed as memory problems (4), difficulties concentrating (5) or problems finding words (6).
[3] The impact on daily activities had to be present during at least 6 months, but not necessarily start within 6 months.
[4] Causes explaining the new occurrence or worsening of a symptom reported by the patient as cause for the symptom (e.g., acute diseases, new comorbidities, flare-up of comorbidity already present, etc.).
[5] Based on an open question asking to report body parts with pain.

Appendix B gives a detailed description of all the criteria of the IDSA definition for PTLDS and the (practical) adaptations made for the HUMTICK study.

Statistical analysis

All analyses were conducted in R version 3.6.3 [33]. Due to the smaller sample size than initially foreseen and as patients and controls were not individually matched, changes were made to the described data analysis in the study protocol [22]. Patient demographics were compared with controls using two-sided Pearson's Chi-squared tests, Fisher's exact tests when appropriate (expected frequency < 5) or t-test for continuous variables.

Comparison of patients vs. controls at follow-up

Both for the comparison of the occurrence of each symptom separately, as for the occurrence of at least one PTLDS-related symptom and the latter combined with impact on daily activities, risk ratios and 95% confidence intervals (CI) were calculated by univariate log-binomial regression analysis. Multivariable analysis could not be performed, as the number of events was too small. Mean standardized questionnaire scores by the LB patient group were compared to controls using linear regression analysis. For the comparisons, at 12 months follow-up, only participants with follow-up were included in the analysis. Other missing data were accounted for using multivariate

imputation by chained equations (20 repetitions) using the MICE package in R [34]. All regression analyses were performed on each of the imputed databases and results were pooled using Rubin's rules, which calculate mean results from all imputed databases and confidence intervals and p-values reflecting the within- and between-imputation variance [34]. Details on the imputation can be found in Appendix C. P-values < 0.05 were considered significant.

PTLDS in LB patients

The binary outcome PTLDS combines different time points following the IDSA definition. Since this definition includes symptom start within 6 months after diagnosis, PTLDS status was known for all patients without any of the six symptoms at T6, even without follow-up at T12. This was also the case for patients that fulfilled the complete PTLDS definition (i.e., including duration 6 months and impact) at T6. PTLDS status could however not be evaluated for patients with symptoms at T6 who did not (yet) fulfill the complete PTLDS definition and had no follow-up at T12 (n EM=3; n DISS=2), as these are still at risk to develop PTLDS. Hence, these patients were censored and the missing status was imputed by the multiple imputation performed (above and Appendix C). Log-binomial regression analysis was used to estimate the proportion of LB patients developing PTLDS, again performed on each imputed database and pooled using Rubin's rules. Disseminated/late LB was analyzed as a risk factor for the development of PTLDS in a log-binomial univariate analysis including all LB patients (EM and disseminated/late LB). Furthermore, within the group of EM patients, associations of other potential risk factors with PTLDS were analyzed i.e., socio-demographic variables, comorbid illness and symptom presence before LB, symptom presence at diagnosis and the treatment prescribed. After univariate log-binomial regression analysis, variables with a p-value < 0.25 were included in a multivariable model on which backward exclusion was performed to retain only significant risk factors (multivariate Wald test, p-values < 0.05). Due to the small sample size and related computational problems, risk factors were not analyzed in the group of disseminated and late LB patients.

Results

Participant characteristics

Informed consent was received from 141 patients with EM, 29 with disseminated/late LB and 157 controls recruited for the study. After exclusion of patients without LB and controls with previous LB, participants without full data at inclusion (i.e., part of questionnaire missing) and participants without follow-up until at least T6 (LTFU), 120 patients with an EM, 15 patients with disseminated/late LB and 128 control persons were included in the baseline analysis of the study. Characteristics of these participants are shown in Table 2. A flowchart for participant exclusion is given in Appendix D. At T12, 94 EM patients, 11 disseminated/late LB patients and 81 controls had follow-up data. As only patients included at the beginning of this study could participate in the follow-up at T24, the number of participants with a follow-up of 24 months was low (25 EM patients and 6 disseminated/late LB patients). Hence, these data were not used for extensive analysis.

Table 2. Characteristics of participants included in the HUMTICK study, Belgium, 2016-2020

Category	EM patients N (%)	DISS patients N (%)	Controls N (%)	p-value EM vs. Controls	p-value DISS vs. Controls
Total	**120**	**15**	**128**	-	-
Gender				0.696	**< 0.001**
Male	46 (38.3)	13 (86.7)	46 (35.9)		
Female	74 (61.7)	2 (13.3)	82 (64.1)		
Age				0.723	0.328
18-29	10 (8.3)	1 (6.7)	14 (10.9)		
30-39	15 (12.5)	0 (0)	15 (11.7)		
40-49	17 (14.2)	4 (26.7)	20 (15.6)		
50-59	39 (32.5)	2 (13.3)	32 (25)		
60-69	23 (19.2)	7 (46.7)	32 (25)		
70+	16 (13.3)	1 (6.7)	15 (11.7)		

Category	EM patients N (%)	DISS patients N (%)	Controls N (%)	p-value EM vs. Controls	p-value DISS vs. Controls
Region				0.520	0.329
Flanders	82 (68.3)	7 (46.7)	81 (63.3)		
Wallonia	34 (28.3)	8 (53.3)	40 (31.2)		
Brussels	2 (1.7)	0 (0)	6 (4.7)		
Not Belgium[1]	2 (1.7)	0 (0)	1 (0.8)		
Highest completed education				**0.017**	0.760
Lower	49 (40.8)	3 (20)	34 (26.6)		
Higher	71 (59.2)	12 (80)	94 (73.4)		
Work				0.414	0.696
Full-time	45 (38.1)	6 (40.0)	59 (46.5)		
Part-time	26 (22)	2 (13.3)	23 (18.1)		
Student	6 (5.1)	0 (0)	5 (3.9)		
Retired	32 (27.1)	6 (40)	36 (28.3)		
Not working, other	9 (7.6)	1 (6.7)	4 (3.1)		
Inclusion period				**0.005**	0.889
Spring/Summer	97 (80.8)	10 (66.7)	83 (64.8)		
Autumn/Winter	23 (19.2)	5 (33.3)	45 (35.2)		
Comorbidity					
Musculoskeletal disease	18 (15.1)	2 (13.3)	10 (7.8)	0.070	0.616
Heart disease	9 (7.6)	0 (0)	4 (3.1)	0.155	1.000
Pulmonary disease	8 (6.7)	1 (6.7)	3 (2.3)	0.126	0.361
Thyroid disorder	7 (5.9)	0 (0)	5 (3.9)	0.470	1.000
Other possibly impacting[2]	10 (8.4)	2 (13.3)	4 (3.1)	0.099	0.121
Other non-impacting[3]	30 (25.2)	3 (20)	26 (20.3)	0.358	1.000
Fibromyalgia, chronic fatigue syndrome	2 (1.7)	0 (0)	1 (0.8)	0.610	1.000
PTLDS-related symptoms in the months before LB or before participation					
Muscle pain	32 (27.1)	1 (6.7)	30 (23.4)	0.506	0.192
Joint pain	37 (31.1)	3 (21.4)	37 (28.9)	0.708	0.757
Fatigue	37 (30.8)	4 (26.7)	44 (34.9)	0.495	0.774
Memory difficulties	14 (11.9)	0 (0)	22 (17.3)	0.228	0.127
Concentration difficulties	15 (12.6)	0 (0)	13 (10.2)	0.559	0.363
Wording difficulties	19 (16.2)	0 (0)	21 (16.5)	0.950	0.363
Any of 6 above	61 (50.8)	6 (40.0)	86 (67.2)	**0.009**	**0.038**
N symptoms[4]	2.5	1.3	1.9	**0.011**	**0.035**

EM: erythema migrans, DISS: disseminated/late Lyme borreliosis, N: number, PTLDS: post-treatment Lyme disease syndrome, LB: Lyme borreliosis

[1] Close to the Belgian border, EM diagnosis by GP in Belgium.
[2] Other diagnosis with impact expected on fatigue, widespread pain or cognitive difficulties: sleep apnea, anemia, cancer within past 2 years, hemochromatosis, Ehler-Danlos, depression, Attention Deficit Disorder, hepatitis, Crohn's disease, chronic pelvic pain.
[3] Other diagnosis with no or limited impact expected on fatigue, widespread musculoskeletal pain or cognitive difficulties, amongst others gastro-esophageal or intestinal diseases, urinary, eye, skin, liver or gynecological diseases.
[4] Mean number of symptoms above, reported by participants with at least one of six symptoms.

Compared to the control group, a significantly higher proportion of EM patients had a lower education (p=0.017) and were included in spring/summer (p=0.005). In the disseminated/late LB group, the proportion of men was significantly higher compared to the control group (p<0.001). None of the other demographics differed significantly between the groups but sample size for disseminated/late LB was very small. While no significant differences were found in the presence of the six PTLDS-related symptoms separately between patients (before LB) and controls (before participation), the proportion reporting any of the six symptoms was higher in control persons compared to LB patients (p=0.009 and p=0.038), whereas the mean number of symptoms, in those with at least one symptom, was higher in EM patients (p=0.011) but lower in disseminated/late LB patients (p=0.035).

Table 3 shows the manifestations of the LB patients at inclusion. Out of the 121 EM patients 63.6% could remember a tick bite with a median of 15 days between the bite and the diagnosis (range 1-102). The median time since first notice of EM equaled 7 days (range 0-90). For the disseminated/late LB group, 7 patients remembered a tick bite but only 4 remembered when it occurred. The median time between start symptoms and diagnosis equaled 32 days (range 4-165). More details on the clinical manifestations in patients with disseminated/late LB can be found in appendix E.

Table 3. Lyme borreliosis manifestations at inclusion, HUMTICK study, Belgium, 2016-2020

	EM patients (i)	Disseminated/late LB (ii)[c]
Baseline	**120**	**15**
EM	115	/[a]
MEM	5	/
LNB early	/	8
LA	/	2
Carditis	/	4[b]
ACA	/	1
+ Possible (alternative scenario)	**5**	**3**
EM	5	/
LNB[c] early	/	2
LNB[c] late	/	1

EM: erythema migrans, MEM: multiple erythema migrans, LNB: Lyme neuroborreliosis, LA: Lyme arthritis, ACA: acrodermatitis chronica atrophicans, LB: Lyme borreliosis

[a] Some patients reported an EM yet no clinical information was available as it was often no longer present at inclusion.

[b] LA was reported in one carditis patient and unconfirmed LNB (without lumbar puncture) was reported in two out of four carditis patients (including the patient with carditis & LA).

[c] No lumbar puncture performed (n=2) or only after treatment & negative (n=1), but patient included by specialist based on clinical symptoms and other laboratory results.

Comparison of patients vs. controls at follow-up

Occurrence of new or worsened non-specific symptoms

Risk ratios for each symptom separately (new or worsened, regularly present since the previous questionnaire) in the patient groups compared to the control group at 6 and 12 months follow-up, are shown in Table 4.

Table 4. New or worsened symptoms in patients with Lyme borreliosis compared to controls at 6 and 12 months follow-up, HUMTICK study, Belgium, 2016-2020

Time point	EM N (%)	DISS N (%)	Controls N (%)	EM vs. Controls		DISS vs. Controls	
				RR (95% CI)	P-value	RR (95% CI)	P-value
Total N							
6 months	120	15	128				
12 months	94	11	81				
PTLDS-related symptoms[1]							
Muscle pain							
6 months	11 (9.2)	2 (13.3)	3 (2.3)	3.91 (1.11-13.77)	**0.034**	5.69 (1.02-31.84)	**0.048**
12 months	7.4 (7.9)	0 (0)	1 (1.2)	6.36 (0.79-51.27)	0.082	0 (0-Inf)	0.997
Joint pain							
6 months	7 (5.8)	5 (33.3)	4 (3.1)	1.87 (0.56-6.25)	0.310	10.67 (3.18-35.83)	**< 0.001**
12 months	11.6 (12.3)	4 (36.4)	6 (7.4)	1.66 (0.64-4.29)	0.296	4.91 (1.61-14.93)	**0.006**
Fatigue							
6 months	15.2 (12.7)	5 (33.3)	4 (3.2)	4.02 (1.36-11.86)	**0.012**	10.55 (3.14-35.44)	**< 0.001**
12 months	8 (8.5)	5 (45.5)	3 (3.7)	2.30 (0.62-8.45)	0.209	12.27 (3.33-45.17)	**< 0.001**
Memory difficulties							
6 months	6 (5.0)	0 (0)	3 (2.3)	2.13 (0.54-8.40)	0.277	0 (0-Inf)	0.995
12 months	6 (6.4)	0 (0)	2 (2.5)	2.59 (0.53-12.60)	0.238	0 (0-Inf)	0.996
Concentration difficulties							
6 months	4 (3.3)	0 (0)	1 (0.8)	4.27 (0.48-38.05)	0.193	0 (0-Inf)	0.997
12 months	5 (5.3)	1 (9.1)	0 (0.0)	Inf (0 - Inf)	0.992	Inf (0 - Inf)	0.997
Wording difficulties							
6 months	2 (1.7)	0 (0)	4 (3.1)	0.53 (0.10-2.88)	0.464	0 (0-Inf)	0.995
12 months	4 (4.3)	1 (9.1)	1 (1.2)	3.45 (0.39-30.69)	0.265	7.36 (0.48-113.68)	0.151
Other symptoms[2]							
Headache							
6 months	5 (4.2)	0 (0)	2 (1.6)	2.67 (0.52-13.59)	0.237	0 (0-Inf)	0.995
12 months	3 (3.2)	0 (0)	2 (2.5)	1.29 (0.22-7.64)	0.776	0 (0-Inf)	0.996
Sensory disorders							
6 months	7 (5.8)	1 (6.7)	1 (0.8)	7.47 (0.92-60.37)	0.059	8.53 (0.55-132.63)	0.125
12 months	3 (3.2)	0 (0)	0 (0.0)	Inf (0 - Inf)	0.995	1.00 (0-Inf)	> 0.999
Night sweats							
6 months	3 (2.5)	0 (0)	3 (2.3)	1.07 (0.22-5.22)	0.936	0 (0-Inf)	0.995
12 months	2 (2.1)	0 (0)	6 (7.4)	0.29 (0.06-1.40)	0.122	0 (0-Inf)	0.993
Excessive sleeping							
6 months	5 (4.2)	0 (0)	3 (2.3)	1.78 (0.43-7.33)	0.424	0 (0-Inf)	0.995
12 months	3 (3.2)	0 (0)	2 (2.5)	1.29 (0.22-7.64)	0.776	0 (0-Inf)	0.996
Difficulties falling asleep							
6 months	2 (1.7)	1 (6.7)	5 (3.9)	0.43 (0.08-2.21)	0.312	1.73 (0.21-14.13)	0.609
12 months	4.8 (5.0)	0 (0)	3 (3.7)	1.36 (0.32-5.74)	0.675	0 (0-Inf)	0.996
Swollen joints							
6 months	2.3 (1.9)	1 (6.7)	2 (1.6)	1.20 (0.17-8.40)	0.850	4.27 (0.40-45.22)	0.226
12 months	2 (2.1)	0 (0)	3 (3.7)	0.57 (0.10-3.40)	0.539	0 (0-Inf)	0.996

EM: erythema migrans, DISS: disseminated/late Lyme borreliosis, N: number, RR: risk ratio, CI: confidence interval, PTLDS: post-treatment Lyme disease syndrome

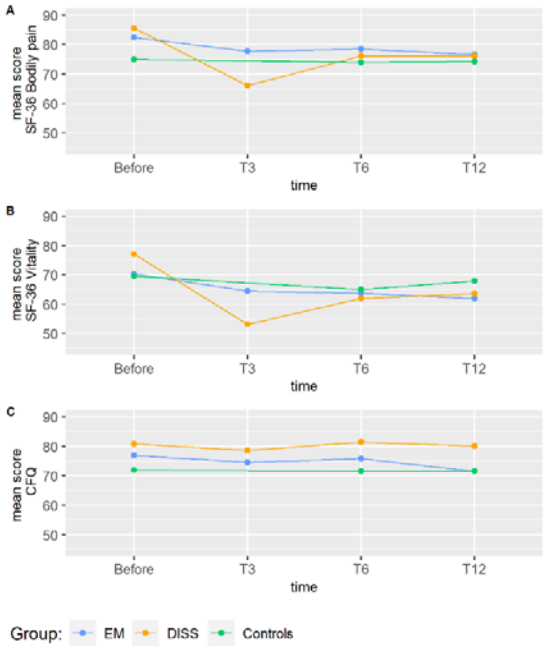
[1] Worsened standardized questionnaire score for symptoms included in definition.

[2] No standardized questionnaire score included in definition.

At 6 months follow-up, new or worsened fatigue and muscle pain were significantly associated with previous LB with a risk ratio of 4.02 (95% CI 1.36-11.86, p=0.012) and 3.91 (95% CI 1.11-13.77, p=0.034) respectively for EM patients and a risk ratio of 10.55 (95% CI 3.14-35.44, p<0.001) and 5.69 (95% CI 1.02-31.84, p=0.048) respectively for disseminated/late LB patients. In the latter, at T6, the risk of joint pain was also significantly higher compared to controls with a RR of 10.67 (95% CI 3.18-35.83, p<0.001). At 12 months follow-up, no symptoms were significantly elevated in EM patients but fatigue and joint pain remained significantly associated with disseminated/late LB with a risk ratio of 12.27 (95% CI 3.33-45.17, p<0.001) and 4.91 (95% CI 1.61-14.93, p=0.006) respectively.

Figure 2 shows the mean scores on the standardized questionnaires and a comparison of scores between patient groups and the control group at each time point (no values for the control group at time T3). A lower score represents more pain, more fatigue or more cognitive difficulties. The SF-36 vitality score at T12 was the only score significantly worse in patients (EM) compared to controls. Disseminated/late LB patients had better vitality before LB compared to controls before participation but not anymore at follow-up, as patients' vitality decreased. Similarly, bodily pain scores were better in LB patients before LB (significant in EM, p=0.007) compared to controls, but the difference was smaller at follow-up (and not significant) for both patient groups. CFQ scores were significantly better in LB patients before start and at T6, but in EM patients not anymore at T12, for disseminated/late LB, CFQ scores remained better than controls at all time points. The most pronounced decline in mean score over time was seen in the disseminated/late LB group at T3 for pain and fatigue with a 23-31% lower score compared to scores before LB.

Figure 2. Mean scores on the standardized questionnaires by group at each time point



Time point	Mean EM	Mean DISS	Mean Controls	p-value EM vs. Controls	p-value DISS vs. Controls
Before	82.4	85.6	75.1	0.007	0.062
T3	77.8	66.1	-	-	-
T6	78.5	76.2	74.0	0.099	0.696
T12	76.6	76.1	74.3	0.471	0.785

Time point	Mean EM	Mean DISS	Mean Controls	p-value EM vs. Controls	p-value DISS vs. Controls
Before	70.4	77.1	69.6	0.711	0.048
T3	64.6	53.3	-	-	-
T6	63.8	62.1	65.1	0.529	0.540
T12	62.1	63.7	68.0	0.016	0.408

Time point	Mean EM	Mean DISS	Mean Controls	p-value EM vs. Controls	p-value DISS vs. Controls
Before	77.1	80.9	72.0	0.001	0.005
T3	74.6	78.7	-	-	-
T6	75.9	81.5	71.6	0.010	0.005
T12	71.6	80.2	71.6	0.975	0.038

All scores can range from 0-100, a lower score represents more pain, more fatigue or more cognitive difficulties (CFQ score is inversed).
Before: before LB (patient groups) or before participation (control group) retrospectively reported at inclusion; T3: 3 months after diagnosis; T6 and T12: 6 and 12 months after treatment, EM: erythema migrans, DISS: disseminated/late Lyme borreliosis.

Any PTLDS-related symptom and impact on daily activities

Table 5 shows the proportion of participants reporting at least one of the six PTLDS-related symptoms as new or worsened, and the proportion of participants that report an additional impact on daily activities. Corresponding risk ratios are shown.

When an impact on daily activities was taken into account, the proportions decreased in all groups by 43-68% depending on the time point and group (Table 5). For this outcome, no statistical significant difference was found between the EM patients and the control group at either 6 or 12 months follow-up. For the disseminated/late LB group, despite the small sample size, both RRs were significant.

Table 5. Any new or worsened PTLDS-related symptom (with impact on daily activities) in patients with Lyme borreliosis compared to controls at 6 and 12 months follow-up, HUMTICK study, Belgium, 2016-2020

Time point	EM N (%)	DISS N (%)	Controls N (%)	EM vs. Controls		DISS vs. Controls	
				RR (95% CI)	P-value	RR (95% CI)	P-value
Total N							
6 months	120	15	128				
12 months	94	11	81				
Any new/worse symptom[1,2]							
6 months	24 (20.0)	7 (46.7)	13.1 (10.2)	1.96 (1.04-3.69)	**0.036**	4.58 (2.15-9.73)	**< 0.001**
12 months	21 (22.3)	7 (63.6)	9 (11.1)	2.01 (0.97-4.16)	0.060	5.73 (2.65-12.39)	**< 0.001**
Any new/worse symptom[1,2] and impact daily activities[3]							
6 months	9 (7.5)	4 (26.7)	4.2 (3.3)	2.27 (0.72-7.19)	0.163	8.07 (2.23-29.22)	**0.002**
12 months	8 (8.5)	4 (36.4)	5 (6.2)	1.38 (0.47-4.08)	0.560	5.89 (1.83-18.98)	**0.003**

EM: erythema migrans, DISS: disseminated/late Lyme borreliosis, N: number, RR: risk ratio, CI: confidence interval

[1] At least one of the following: muscle pain, joint pain, fatigue, memory difficulties, concentration difficulties or difficulties finding words.

[2] Symptom reported as regularly present since the previous questionnaire, worsened compared to health before Lyme borreliosis and worsened score on standardized questionnaire for the symptom present (past 4 weeks).

[3] Limited on the modified GALI and having at least slight problems with daily activities on the standardized questionnaire (EQ-5D-5L, 3th question, past 4 weeks).

PTLDS in LB patients

Twelve EM patients were excluded from the PTLDS-analysis as they reported a history of unexplained or undiagnosed extreme fatigue, widespread musculoskeletal pain or cognitive difficulties or were previously diagnosed with fibromyalgia or chronic fatigue syndrome.

After combining new or worsened symptoms and impact on daily activities at the different time points, in order to include the time frame proposed by the IDSA (Appendix B), the estimated proportion of LB patients developing PTLDS equaled 5.9% (95% CI 2.7-12.9) in EM patients and 20.9% (95% CI 6.8-64.4) in disseminated/late LB patients (Table 6). Characteristics of PTLDS patients (non-imputed) are shown in Appendix F. Three EM patients (2.8%) fulfilled the complete PTLDS definition at T6 as

symptoms impacting daily activities were present during the first 6 months after treatment, one of which still at T12. The other three EM patients with PTLDS fulfilled the complete definition only at T12, their symptoms started before T3 or T6 but were not yet present during 6 months or did not yet impact daily life activities at 6 months follow-up, but did afterwards. In the group of disseminated/late LB patients, three patients had PTLDS at T6 (20.0%) of which 2 still at T12. For none of the patients, an explanatory cause was reported for all PTLDS-related symptoms.

Risk factors for the development of PTLDS

In a univariate analysis including all LB patients, the risk of PTLDS was higher for the disseminated/late LB group compared to the EM group with a RR of 3.53 (95% CI 0.98-12.68, p=0.053), borderline non-significant. For other risk factors, within the EM group, region, pulmonary disease and joint pain before LB, number of symptoms other than EM and having multiple EM at diagnosis had a p-value < 0.25 in the univariate analysis (Appendix G). After backwards exclusion from the multivariable analysis, none of the variables were significant.

Alternative scenarios PTLDS

Estimating the incidence of PTLDS using different inclusion criteria or without imputation provided similar results than the baseline scenario (Table 6 and Appendix H). When a definition with higher sensitivity was used for new or worsened symptoms, namely without inclusion of a worse standardized questionnaire score as a criterion, the proportion of PTLDS in EM patients increased to 8.7% (95% CI 4.7-16.4).

Table 6. Incidence of post-treatment Lyme disease syndrome (PTLDS) using different inclusion or outcome criteria, HUMTICK study, Belgium, 2016-2020

	EM patients		DISS patients	
	PTLDS[1]/N	Proportion (95% CI)	PTLDS[1]/N	Proportion (95% CI)
Baseline	6.4/108	5.9 (2.7-12.9)	3.15/15	20.9 (6.8-64.4)
1: Possible cases included	6.4/113	5.6 (2.6-12.3)	4.15/18	23.0 (9.0-58.8)
2: Insufficiently treated excluded	5.4/104	5.2 (2.2-12.2)	3.15/14	22.4 (7.3-69.0)
3: No standardized questionnaires	9.45/108	8.7 (4.7-16.4)	3.25/15	21.5 (7.0-66.0)
4: No imputation	6/105	5.7 (2.6-12.4)	3/13	23.1 (8.6-62.3)

EM: erythema migrans, DISS: disseminated/late Lyme borreliosis, PTLDS: post-treatment Lyme disease syndrome, N: number, CI: confidence interval

[1] The decimals in the numbers of PTLDS cases are the result of imputing the outcome in the censored patients, with having PTLDS in only part of the 20 repetitive imputations.

Discussion

Several studies have investigated non-specific persistent symptoms after antibiotic treatment for LB, but only few were prospective cohort studies including a non-LB control group [4–6,35–37]. Furthermore, only few studies have applied the full case definition for PTLDS even though it has been proposed more than a decade ago by the IDSA [16]. In addition, the definition is still open for interpretation and the methods used to evaluate symptoms and impact after LB in practice vary widely [25,38]. In this prospective study, extensive efforts were made to apply the different components of the definition to identify patients with PTLDS. To allow comparison with previously reported results and to show the impact of the definitions used, different steps were followed to present and discuss the study results. First, we compared the occurrence of several new or worsened symptoms as such between LB patients and controls at 6 and 12 months follow-up. Second, the occurrence of at least one of the symptoms listed in the PTLDS definition was compared between LB patients and controls at the same time points, and an impact on daily activities was added. Third, we estimated the proportion of patients developing PTLDS in EM patients and the disseminated/late LB

patients, according to the full IDSA case definition by combining different follow-up points to include the time frame proposed.

In the separate symptom analysis in our study, only the risk of fatigue and pain were significantly higher in LB patients compared to controls. Although multiple studies have reported high frequencies of cognitive difficulties after LB [11,15,39], this was not the case in our study.

Following the IDSA definition for PTLDS, this study further looked at the occurrence of either widespread musculoskeletal pain, fatigue or cognitive difficulties. When impact on daily activities was not taken into account, as done in most previous studies in literature, the risk of any of these symptoms was significantly higher in EM patients compared to controls at 6 months follow-up, with a 10% higher proportion of EM patients reporting any new or worsened symptom compared to controls (20.0% vs 10.2% respectively). Three prospective European studies that reported new or worsened symptoms without impact, yet excluding other causes, reported a somewhat lower symptom proportion of 4.6-10.7% in solitary EM patients and 15.9% in MEM patients at 6 months follow-up and 2.2-7.3% at 12 months follow-up respectively [4–6]. MEM has previously been identified as a risk factor for non-specific symptoms after LB [6–8]. On the other hand, a study in the US reported a higher proportion compared to our results, with 36% of patients reporting new-onset subjective symptoms at 6 months follow-up, yet they included EM patients with systemic symptoms only [11,38]. Since LB is caused by different genospecies in Europe and America, a difference might be observed in the occurrence of PTLDS between European and American patients [1,40].

When an impact on daily activities was taken into account, our study did not find a significant difference between EM patients and controls in the occurrence of any of the PTLDS-related symptoms at either 6 or 12 months follow-up, indicating an overall favorable evolution after treatment for EM. In general, the proportions of patients and

controls reporting symptoms were greatly reduced by the addition of the impact as a criterion in the analysis at the separate time points, namely with 43-68% (Table 5), showing the impact of the definitions used. A few prospective studies that compared non-specific symptoms in EM patients to a non-LB control group (without considering impact on daily life), did not report significant differences at follow-up [4–6,35,36]. On the other hand, Ursinus et al. (2021) did found significant differences in the prevalence of persistent symptoms between EM patients and controls in a large prospective study recently conducted in the Netherlands [37]. More specifically, they defined persistent symptoms as impaired scores on standardized questionnaires for fatigue, pain or cognitive impairment, during at least 6 months, with onset < 6 months and found the prevalence of these persistent symptoms to be 3.9% and 6.0% higher in EM patients compared to two different control groups respectively (general population and persons with a tick bite without LB) [37]. Nevertheless, comparison of the results of different studies is challenging as different outcomes are assessed (e.g., prevalence, standardized questionnaire scores, new/worse symptoms, etc.) and different study populations, study designs and follow-up periods are used.

In the group of disseminated/late LB patients, our study did find a significantly higher risk on any PTLDS-related symptom compared to controls, both with or without taking into account impact on daily activities. The study by Ursinus et al. (2021) also reported a significant difference between disseminated LB patients and controls, with a prevalence of persistent symptoms that was 11.0% and 13.1% higher in patients compared to the two control groups respectively [37]. Significant differences in non-specific symptoms between LNB patients and controls have also been described elsewhere [15]. The 36.5% higher proportion of symptoms without impact in this group compared to the controls at 6 months follow-up in our study, is also in accordance with higher proportions of symptoms that have been reported in literature, such as a mean of 28% in a review of 44 studies in LNB [13]. Other studies have also identified

dissemination of LB as a risk factor for the development of persisting symptoms compared to EM [6,41].

In general, a high background prevalence of PTLDS-related symptoms was present in our study with 51% of EM patients, 40% of disseminated/late LB patients and 67% of controls reporting at least one of the six PTLDS-related symptoms in the months before LB onset or before participation (Table 2). This prevalence differed significantly between LB patients and controls. Similarly, several mean standardized questionnaire scores were better in the patient cohorts before LB compared to controls before participation. It needs to be noted that, for the patients, these pre-Lyme prevalences and scores were reported retrospectively at inclusion (when current LB symptoms were possibly present) for the period before LB started, and recall bias might have occurred, especially in disseminated/late LB patients who were sometimes not included until a few weeks after the start of treatment. On the other hand, it is possible that LB patients are somewhat more active than the general population, as tick bites often occur during active outdoor activities. In order to increase specificity and identify patients with symptoms possibly related to LB, this study focused on symptoms that were new or worsened at follow-up only. This way, the study assessed a subset of patients less influenced by the high background prevalence of PTLDS-related symptoms. Other studies analyzing overall prevalence in patients and controls, also found proportions as high as 70-85% experiencing one of the symptoms assessed at baseline or follow-up [5, 6, 42].

After applying exclusion criteria proposed by the IDSA, as well as the time frame of symptom onset within 6 months and presence during at least 6 months (Figure 1 and Appendix B) this study estimated the proportion of PTLDS to be 5.9% (95% CI 2.7-12.9) in EM patients and 20.9% (95% CI 6.8-64.4) in disseminated/late LB patients. Belonging to the latter group increased the risk of PTLDS with a RR of 3.53 (95% CI 0.98-12.68, p=0.053). To our knowledge, no study has previously looked at the occurrence of new or worsened symptoms, including the impact on daily activities and the time frame as

criteria for identification of PTLDS cases as was done in our study. The three prospective European studies above reported no cases of PTLDS at 12 months follow-up as there was no reduction in previous activity levels. Yet, it seems that the latter was not assessed at 6 months follow-up [4–6]. Two prospective studies in the US reported 5.6% and 11% PTLDS in EM patients with systemic symptoms (flu-like symptoms or dissemination EM) but they were followed-up for 6 months only [35,38]. In our study, only three out of 108 EM patients (2.8%) fulfilled the complete PTLDS definition at 6 months follow-up, yet 5.9% did at T6 or T12, highlighting the importance of longer follow-up and combination of different follow-up points in PTLDS research to allow proper inclusion of the time frame proposed by the IDSA. It needs to be noted that newer clinical guidelines of the IDSA published in 2021 do not include a definition for PLDS or PTLDS to avoid its use in clinical settings. The definition was previously also only proposed for research purposes [25,43].

No significant risk factors could be identified in the study, yet the analysis was limited by the small number of PTLDS cases found. Previous studies have reported MEM, delay in treatment, more symptoms at diagnosis, some specific symptoms at diagnosis such as fever, headache, neck stiffness but also older age, female gender, more comorbidities or fatigue in the past year, as a risk factor for post-treatment symptoms in LB patients, yet not always confirmed by other studies [6–8,41,44–48]. Further research with larger sample sizes is needed. To date, also the cause of PTLDS remains unknown, one of the hypotheses is that it is a post-infectious syndrome as seen with other infectious diseases such as Epstein-Barr virus and Q-fever and now also COVID-19 [49,50]. More research into long-COVID that will be performed might contribute to the understanding of the pathophysiology of these post-infectious syndromes, yet it remains unknown if they share a common mechanism.

Important strengths of the current study are the prospective design, the follow-up of a control group, the use of standardized questionnaires, the assessment of health before Lyme borreliosis or participation (although retrospective) and the analysis of

different outcomes and alternative scenarios. The latter allow to assess the impact of changing the case definition and study population on the results. In these, inclusion of possible cases for the PTLDS analysis didn't change the results a lot yet it concerned only few additional patients (Appendix H). A review in LNB patients reported more residual symptoms in studies including possible cases [13]. Also when only sufficiently treated patients were retained from the baseline PTLDS analysis, as proposed by the IDSA definition, the proportions of patients with PTLDS did not differ significantly compared to the baseline analysis (Appendix H). Including these patients is closer to the real life situation where patients are still not treated optimally, as was observed in our study. Even though treatment recommendations were provided to the GPs, a high number of patients (35.6% of EM patients in the PTLDS analysis) was treated with a higher dose or longer period than advised in Belgian guidelines [51].

There are also some limitations to this study. For all groups, predefined sample sizes could not be included [22]. There were unexpected difficulties, mainly in recruiting enough patients with well documented confirmed disseminated/late LB. As a consequence, the sample size of this group was small which causes confidence intervals to be large and increases the risk for significant effects to be overestimated in effect size [52]. These difficulties with inclusion can be due to strict case definitions used, in which different laboratory results need to be available. Indeed, for three possible LNB cases, cerebrospinal fluid analysis was not available or it was performed after treatment. The limited sample size also precluded confounders to be added to the analysis. When age and gender were added in the EM group, same symptoms remained significant, yet any new/worse PTLDS related symptom was also significant at T12 ($p=0.04$, results not shown). Furthermore, follow-up at T12 was missing for several patients which eventually resulted in a missing PTLDS status for three EM patients and two disseminated/late LB for which multiple imputation was performed. At T24, data were only available from few patients. All results are based on patient reports only, there was no involvement of a GP or study investigator in the patient follow-up. A

recent prospective study in EM patients found the proportion of patients with PTLDS-symptoms to be higher when based on clinical assessment compared to survey data only [36], yet another study found good sensitivity for a standardized questionnaire method used in their study, however it lacked specificity [53]. Since the PTLDS definition comprises the exclusion of symptoms caused by another illness, participants were asked to report a cause, if known, of their symptoms. However, this response was often missing and anyhow, it is difficult for a non-medical person to assess. Therefore, it is possible that some symptoms might have been related to another cause and were wrongly classified as PTLDS. It is also possible that some control persons would still fulfill the definition of PTLDS (without previous LB), yet this could not be checked in the current study as no data at T3 were available. Furthermore, despite the inclusion of a control group, one could never account for a possible psychological effect of receiving a certain diagnosis, such as Lyme borreliosis, on the development of symptoms after treatment in the LB group, as opposed to the control group.

Conclusions

In our study, PTLDS was present in both LB cohorts, yet with a higher percentage in patients with disseminated/late LB. To our knowledge, this is the first study that allowed to estimate the proportion of PTLDS as defined by the IDSA (2006), including strict criteria on symptoms, impact on daily activities and the time frame. Since the proportions of patients with non-specific symptoms after LB changed substantially when taking into account the impact of symptoms on daily activities and the time criterion, future research should include these different components. Yet, development and validation of standardized methods that are easily applicable in practice to assess these components is of great importance. As symptoms are highly prevalent in the general population and occur without being disabling, inclusion of a non-LB control group is also essential in these future studies. In order to have a sufficiently large

sample size, allowing identification of risk factors and possible causes for PTLDS, international collaborations should be encouraged.

Ethics approval and consent to participate

Informed consent was obtained from all study participants. Ethical approval was obtained from the "Comité d'Ethique Hospitalo-Facultaire Saint-Luc UCL" (ref.: 2016/13AVR/166) which served as the principal ethical committee for the study and from the ethical committee of each participating hospital (UZ Leuven, Ziekenhuis Oost-Limburg, CHU UCL Namur - site Godinne, CHU de Liège, Clinique Saint-Pierre Ottignies, CHR Namur). The study has been approved by the Commission to Protect the Personal Privacy (Beraadslaging Nr. 16/038, reference: SCSZG/16/144).

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Appendices

Table of contents

Appendix A

Table A1. Case definitions for inclusion in the HUMTICK study, Belgium, 2016-2020 [1]

Cohort	Case definition
Cohort 1: EM	- Clinical presentation of EM or MEM, confirmed by a GP
Cohort 2: Confirmed disseminated Lyme borreliosis	- Positive polymerase chain reaction (PCR) or culture OR - Positive serology AND at least one clinical manifestation compatible with disseminated Lyme borreliosis (confirmed by the treating physician) [12]. [see below for the specific description of this case definition]

Specific description of the case definitions for (confirmed) cases of disseminated Lyme borreliosis which will be included in the HUMTICK study:

Adapted from CDC and EUCALB case definitions* [2,3]

Laboratory evidence:

- Isolation or positive PCR from tissue or body fluid

OR

- Positive serology (using the two-tier ELISA and Western Blot)

OR

- Pleocytosis and antibody production against *B. burgdorferi* in the cerebrospinal fluid (CSF), evidenced by a higher titer of antibody in CSF than in serum (positive antibody index) (always necessary for Lyme neuroborreliosis) [4]

AND clinical evidence:

at least one of the following clinical manifestations corresponding with disseminated Lyme borreliosis, when an alternate explanation is not found:

- **Skin manifestation:**
  Multiple erythema migrans (included in cohort 1 in the current study) or acrodermatitis chronica atrophicans.
- **Musculoskeletal system (Lyme arthritis):**

Recurrent episodes (weeks or months) of objective joint swelling in one (commonly the knee) or a few joints, sometimes followed by chronic arthritis in one or a few joints. The following manifestations are not considered for inclusion: chronic progressive arthritis not preceded by brief attacks, chronic symmetrical polyarthritis, and arthralgia, myalgia, or fibromyalgia syndromes alone.

- **Nervous system (neuroborreliosis):**

  Any of the following neurological symptoms (alone or in combination): lymphocytic meningitis; cranial neuritis, particularly facial palsy (uni- or bilateral); radiculitic pain; or, rarely encephalomyelitis. Headache, fatigue, paresthesia, or mildly stiff neck alone, are not considered for inclusion.

- **Cardiovascular system (carditis):**

  Acute onset of high-grade (2nd-degree or 3rd-degree) atrioventricular conduction defects that resolve in days to weeks and are sometimes associated with myocarditis. Palpitations, bradycardia, bundle branch block, or myocarditis alone are not considered for inclusion.

* Belgium is an area endemic for Lyme Borreliosis; therefore, all included patients fulfill the criterion of exposure to *B. burgdorferi.*

Appendix B

Table B1. Definition post-treatment Lyme disease syndrome according to the Infectious Diseases Society of America (IDSA) and HUMTICK project.

Theoretical definition PTLDS	
IDSA (2006)	**HUMTICK**
'Documented episode of early or late Lyme disease, case definition of the Centers for Disease Control and Prevention'	'Confirmed Lyme disease based on case definitions' (Appendix A)
'Treatment with generally accepted regimen with resolution/stabilization of objective manifestations'	'Treatment prescribed by participating GP or specialist'
Subjective PTLDS symptoms:	
'Onset of any of the following: - fatigue, - widespread musculoskeletal pain, - complaints of cognitive difficulties'	Onset **or worsening** of any of the following 6 symptoms: fatigue (1); widespread musculoskeletal pain expressed as: muscle pain (2), joint pain (3) at more than one place of the body; cognitive difficulties expressed as: memory problems (5), difficulties concentrating (6) or problems finding words (7)
'Symptom onset within 6 months of the diagnosis of Lyme disease and persistence of continuous or relapsing symptoms for at least a 6 month period after completion of antibiotic therapy'	'Symptom onset within 6 months of the diagnosis of Lyme disease and regularly present for at least 6 months after antibiotic therapy' = Onset before T3 and regularly present up and until T6 (and possibly also between T6 and T12) OR Onset before T6 and regularly present between T6 and T12
Impact daily activities:	
'Subjective symptoms are of such severity that, when present, they result in **substantial reduction in previous levels of occupational, educational, social, or personal activities**.'	'Subjective symptoms are of such severity that they impact daily activities (e.g., work, school, hobbies or other). Start of impact did not necessarily had to coincide with start symptoms, it could start later but needed to last 6 months'.
Exclusion	
A diagnosis of an underlying disease or condition that might explain the patient's symptoms	If a patient reports another cause, which explains the new occurrence or worsening of a symptom (e.g., acute disease, newly diagnosed disease, flare-up of existing disease, etc.) the symptom is not included as new or worsened symptom for the final PTLDS
A diagnosis of fibromyalgia or chronic fatigue syndrome before the onset of Lyme disease.	Previously diagnosed fibromyalgia, chronic fatigue syndrome or chronic Lyme disease
A prolonged history of undiagnosed or unexplained somatic complaints, such as musculoskeletal pains or fatigue, before the onset of Lyme disease.	A history of unexplained or undiagnosed complaints such as extreme fatigue, widespread musculoskeletal pain or cognitive difficulties.

An active, untreated, well-documented coinfection such as babesiosis	EM patients were tested for other tick-borne pathogens at diagnosis, yet only with PCR. None were positive (personal communication).
The presence of objective abnormalities on physical examination or on neuropsychologic testing that may explain the patient's complaints.	No additional examination or testing carried out at follow-up in the scope of this study.
Laboratory or imaging abnormalities that might suggest an undiagnosed process distinct from post–Lyme disease syndrome	No additional examination or testing carried out at follow-up in the scope of this study.
Although testing by either culture or PCR for evidence of *Borrelia burgdorferi* infection is not required, should such testing be done by reliable methods, a positive result would be an exclusion.	No additional examination or testing carried out at follow-up in the scope of this study.

Appendix C

Missing data

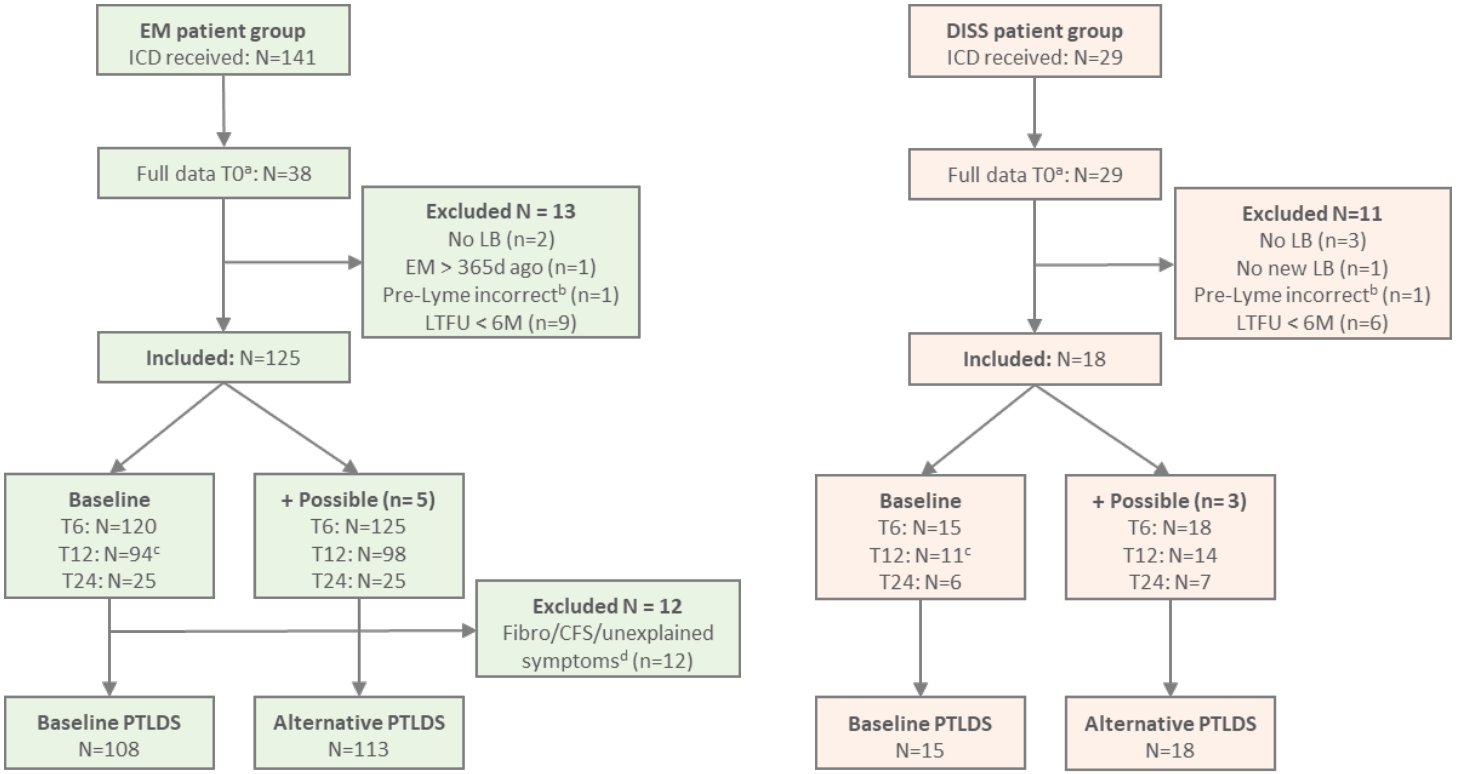
For the baseline analysis, the proportion of missing data ranged between 0 and 2.5% for new or worsened symptoms in the separate symptom analysis (Table 4) and between 0 and 1.7% for any of the 6 PTLDS-related symptoms (without or combined with impacting on daily activities) (Table 5). The final PTLDS status was known for all patients with follow-up until T12 as well as for those with follow-up until T6 without any of the 7 symptoms present at that time as the latter are not at risk to develop PTLDS anymore since symptoms should have started within 6 months after treatment. In the end, PTLDS status was missing for 3 EM patients (2.8%) and 2 disseminated/late LB patients (13.3%).

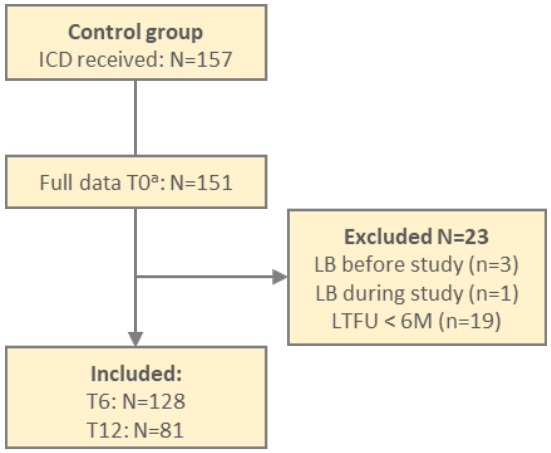
As missing outcome variables were not missing completely at random (MCAR) due to conditional questioning and the definitions used (combining multiple questions with and/or), e.g., PTLDS status was only missing when the person did experience symptoms at T6, they could not be deleted. Instead, missing data were accounted for under the assumption of missing at random, i.e., depending on other observed data [5]. In a first step, existing rules on handling missing values to calculate the standardized questionnaire scores were applied: for the SF-36 bodily pain (2 items), if the first item is missing the second is recoded slightly different and the missing item takes the same value as the none missing item. For the SF-36 vitality subscale (4 items), missing items are imputed with the mean of the non-missing items, provided that not more than half of the items are missing. If more than half are missing, scale score remains missing. As for CFQ no guidelines were available, the same method as used for the SF-36 vitality scale was applied. In a second step, multivariate imputation by chained equations (package mice in R [6]) was used to impute remaining missing scale scores and other missing values based on other variables related to PTLDS at diagnosis and follow-up until T12 (including outcome variables, variables related to the symptoms, daily

activities, quality of life, etc., as well as possible risk factors). The imputation was performed on databases for EM patients (n=125, including possible cases), disseminated/late LB patients (n=18) and control persons (n=128) separately as different variables were collected (different questionnaires). Default imputation methods were used: predictive mean matching for continuous variables, logistic regression for binary variables and multinomial logit models for categorical variables with more than 2 categories. Due to the high number of variables, to avoid computational problems, the imputation model was restricted to select about 15 predictors for each variable (range 8-21) as advised by Van Buuren et al. (2011) [6]. Predictors were chosen based on highest correlation excluding some duplicate information, taking into account the proportion of usable cases, when < 0.25, predictors were excluded (Minpuc = 0.25). Due to the latter, variables of T12 for which many were missing due to missing follow-up, were not used to predict each other. Additionally, by default, the mice algorithm leaves out multicollinear or constant variables as predictors of the imputation process. Post-processing was used to avoid implausible values by selecting closest plausible value. In the end, twenty multiple imputed data sets were generated performing 20 iterations. Full questionnaire data missing at T3 (n=3) or T12 (n=27) were not used in the analysis.

Appendix D

Figure D1: flowchart patient inclusion





EM: erythema migrans, ICD: Informed Consent Document, DISS: disseminated/late Lyme borreliosis, LB: Lyme borreliosis, T0: Inclusion, LTFU: Loss to follow-up, T6: 6 months after treatment, T12: 12 months after treatment, Fibro: fibromyalgia, CFS: Chronic Fatigue Syndrome

[a] Exclusion only if complete or complete part of questionnaire T0 was missing (e.g., GP part, patient part or standardized questionnaire part).

[b] One EM patient reported chronic LB as a comorbidity due to a LB infection years ago and assessed pre-Lyme health erroneously as his/her health before that LB diagnosis. One disseminated LB patient assessed his health before LB as his health before a rheumatoid arthritis diagnosis causing similar symptoms.

[c] Of the 125 EM patients at T6, 13 did not respond at T12 (true LTFU) and 14 were not followed-up as they started to participate late in the study. For disseminated/late LB, all patients responded at T12, 4 patients were not followed-up as they were included late. For the control group, 14 did not respond, whereas 32 were not followed-up.

[d] History of unexplained or undiagnosed extreme fatigue, widespread musculoskeletal pain or cognitive difficulties.

Appendix E

Table E1: Manifestations of patients with disseminated/late LB at inclusion

	Age	Gender	Main manifestation (diagnosed by specialist)	Symptoms reported by patient	Days start symptoms to TM	Time bite to TM	Hosp.
Confirmed cases (baseline analysis)							
#1	65-69	M	Early LNB: • (Meningo)radiculitis (thoracic spinal nerves T4 → T7)	Intense pain (right side chest), sensory disorders (legs), sleep disorders, mood disorders, fatigue, muscle pain, red spot/circle (place suspected tick bite)	32	Unk	No
#2	65-69	M	Early LNB: • (Meningo)radiculitis (cutaneous nerves, sciatic nerve and scalp) • Meningitis	Sensory disorders (arms, neck, scalp), headache, neck stiffness, joint pain (wrists, elbows, neck, shoulder)	75	Unk	Yes
#3	25-29	M	Early LNB: • (Meningo)radiculitis (lumbar and popliteal pain) • Other : nuchalgia, paresthesia, dysesthesia	Intense pain (cervicals, lower back, behind the knees), sensory disorders (pelvis, legs), headache, sleep disorders, mood disorders, night sweats (with waking up), fever (regular), neck pain, fatigue	27	34	Yes
#4	65-69	M	Early LNB: • (Meningo)radiculitis (cervical spinal nerve C2, bilaterally) • Meningitis	Intense pain (scalp), loss of strength (left lower limb, right hand), mood disorders, fatigue, abdominal bloating	25	Unk	Yes
#5	60-64	M	Early LNB: • (Meningo)radiculitis (n. abducens)	Double vision, headache, sleep disorders, fatigue, joint pain (leg), muscle pain	34	49	Yes
#6	60-64	M	Early LNB: • Peripheral facial palsy	Paralysis (left side face), back pain	18	Unk	Yes
#7	55-59	F	Early LNB: • Meningitis • Cranial nerve loss (n. abducens left, n. oculomotorus left)	Paralysis (left eye nerves), troubled vision, headache, fever (1x), neck stiffness, fatigue	10	Unk	Yes
#8	50-54	M	Early LNB: • Peripheral facial palsy • Other: diffuse allodynia body	Intense pain (back, abdomen), paralysis (face), sensory disorders (head to upper legs), joint pain (shoulders, hips, back), sleep disorders (due to pain), neck stiffness, fatigue, muscle pain	18	Multiple tick bites	Yes
#9	45-49	M	Lyme arthritis: • Right knee	Joint pain (right knee), joint swelling (right knee), heart palpitations (sporadically), multiple red spots/circles[a] (back, not place tick bite)	89 (joints) 14 (MEM not confirmed[a])	89-118[b]	No

#10	70-74	F	Lyme arthritis: • Left knee	Joint pain (left knee), joint swelling (left knee), (multiple) red spot (left shin, place tick bite) [a]	50 (EM not confirmed[a]) 4 (joints)	51	No
#11	65-69	M	Acrodermatitis chronica atrophicans	Joint pain (hand), joint swelling (hand), loss of strength (hand), skin lesion (hand)	165	4-5 years[b]	No
#12	40-44	M	Lyme carditis Lyme arthritis *Lyme neuroborreliosis (not confirmed, no CSF)* • *(Meningo)radiculitis (lumbar spinal nerve L5 and ulnar nerve)*	Heart palpitations, shortness of breath, joint pain (foot, knee, elbow), joint swelling (foot), intense pain (elbow), loss of strength (right hand), mood disorders, multiple red spots/circles[a] (foot/ankle), night sweats (with waking up), muscle pain	45	Unk	Yes
#13	40-44	M	Lyme carditis	Heart palpitations, shortness of breath, muscle pain, red spot/circle[a] (place tick bite)	46 (MEM not confirmed[a]) 2 (heart)	47	Yes
#14	60-64	M	Lyme carditis	Heart palpitations, night sweats (with waking up), fatigue	4	Unk	Yes
#15	40-44	M	Lyme carditis *Lyme neuroborreliosis (not confirmed, no CSF)* • (Meningo)radiculitis (sacral spinal nerve S1 right)	Shortness of breath, joint pain (left wrist, lumbar), fever (regular), fatigue, muscle pain, red spot/circle (place suspected tick bite)	42 (EM not confirmed[a]) 20 (heart)	Unk	Yes
Possible cases (alternative analysis)							
#16	55-59	M	*Early LNB (not confirmed, no CSF)* • *Peripheral facial palsy* • *Other cranial nerve loss (trigeminal nerve V)*	Paralysis (right side face), sensory disorders (scalp), fatigue, flu-like state	15	Unk	No
#17	45-49	M	*Early LNB (not confirmed, CSF only after treatment, no IgG/IgM)* • *(Meningo)radiculitis (lumbar spinal nerve L5 right)*	Intense pain (right lower limb), slower reactions/reflexes, night sweats (with waking up), fever (2x), neck stiffness, fatigue, muscle pain, joint pain	61	Unk	No
#18	40-44	M	*Late LNB (not confirmed, no CSF)* • *Meningoencephalitis* • *Radiculomyelitis (lumbar spinal nerve L5 bilateral, sacral spinal nerve S1 left)*	Intense pain (lower back, right foot), loss of strength (right foot & toes), sensory disorders (right foot), slower reactions/reflexes, fatigue, mild cognitive difficulties	843	840-901[b]	No

F: female; M: male; TM: treatment; hosp.: hospitalization; LNB: Lyme neuroborreliosis; unk: unknown; EM: erythema migrans; MEM: multiple erythema migrans

[a] Reported by patient but not present at diagnosis, so impossible to confirm diagnosis erythema migrans or multiple erythema migrans by medical specialist.

[b] Range given by patient.

Appendix F

Table F1. Characteristics of patients with PTLDS (not-imputed only), HUMTICK study, Belgium, 2016-2020

PTLDS case	Age	Gender	T0	PTLDS[1]	New/worse PTLDS related symptoms[2]	Symptom start	Symptom duration	Impact start	Impact duration	Other symptoms at follow-up
#1	50-54	F	EM	**≤T1-24** (end FU)	Muscle pain, joint pain (T3), fatigue (T3, T12), memory (T3, T6, T12, T24), wording (T3, T12, T24), concentration (T12)	≤T1	24m (end FU)	≤T1	24m (end FU)	Excessive sleeping (T12)
#2	55-59	F	EM	**≤T1-6**	Fatigue (T3, T6, T12), muscle pain, joint pain (T12)	≤T1	12m (end FU)	≤T1	6m	Headache (T3), Night sweats (T3), difficulties falling asleep (T12)
#3	55-59	F	EM	**≤T1-6**	Muscle pain (T3, T6), joint pain (T3=NA, T6) severity fatigue = NA (T3, T6)	≤T1	6m	≤T1	6m	Sensory disorders (T6), Night sweats (T6, T3 severity=NA) Excessive sleeping (T6, T3 severity=NA)
#4	60-64	M	EM	**T6-12**	Muscle pain (T6, T12), joint pain (T3, T12) memory (T6), wording (T6)	≤T1	12m	T6	6m	Headache (T3), difficulties falling asleep (T3, T12)
#5	50-54	M	MEM	**T6-12**	Fatigue (T6, T12) Memory, wording, concentration (T12)	T3	21m (end FU)	T6	6m	Excessive sleeping (T6, T12), Sensory disorders T12=NA, difficulties falling asleep T12=NA
#6	50-54	M	EM	**T6-12**	Fatigue (T6, T12), memory (T6)	T3	9m	T6	6m	Headache (T6), sensory disorders (T6)
#7	50-54	M	Early LNB	**≤T1-24** (end FU)	Fatigue (T3, T6, T12, T24), joint pain (T3, T24)	≤T1	24m (end FU)	≤T1	24m (end FU)	/
#8	45-49	M	LA	**≤T1-T12** (end FU)	Fatigue (T3, T6, T12) muscle pain (T6), joint pain (T12)	≤T1	12m (end FU)	≤T1	12m (end FU)	Initial swollen knee, until T6, initial joint pain knee (T3, T6, T12)
#9	25-29	M	Early LNB	**≤T1-6**	Fatigue (T3, T6, T12)	≤T1	12m (end FU)	≤T1	6m	Neurological pain multiple locations (T3), one location (T6, T12)

PTLDS: post-treatment Lyme disease syndrome, F: female, M: male, EM: erythema migrans, MEM: multiple erythema migrans, LNB: Lyme neuroborreliosis, LA: Lyme arthritis, T0: diagnosis, T3: 3 months after diagnosis; T6, T12 and T24: 6, 12 and 24 months after treatment, NA: missing, FU: follow-up, m: months

[1] Period for which both the symptom and impact criterion are fulfilled, both based on general question and standardized questionnaire/question.

[2] Muscle pain or joint pain at more than 1 location, fatigue, memory problems (forgetfulness), difficulties concentrating or problems finding words, without other cause reported.

Appendix G

Table F1. Associations of possible risk factors with the development of post-treatment Lyme disease syndrome, HUMTICK study, Belgium, 2016-2020 - Univariate log-binomial analysis

Category	N[1]	PTLDS N (%)	RR (95% CI)	P-value
Total	**108**	**6.4 (5.9)**		
Gender				
Male	45	3.0 (6.7)	Reference	-
Female	63	3.4 (5.4)	0.80 (0.17-3.81)	0.779
Age				
Years			1.01 (0.96-1.06)	0.681
Region[2]				0.183**
Flanders	77	2.4 (3.1)	Reference	-
Wallonia	29	4.0 (13.8)	4.52 (0.88-23.20)	0.071
Brussels	2	0.0 (0.0)	0.00 (0.00-Inf)	0.996
Highest completed education				
Lower	46	3.4 (7.4)	Reference	-
Higher	62	3.0 (4.8)	0.66 (0.14-3.14)	0.600
Work				0.889
Full-time	44	2.4 (5.5)	Reference	-
Part-time	24	3.0 (12.5)	2.34 (0.42-12.99)	0.328
Student	4	0.0 (0.0)	0.00 (0.00-Inf)	0.998
Retired	28	0.0 (0.0)	0.00 (0.00-Inf)	0.995
Not working, other	8	1.0 (12.5)	2.34 (0.24-23.05)	0.463
Inclusion period				
Spring/Summer	88	5.4 (6.1)	Reference	-
Autumn/Winter	20	1.0 (5.0)	0.82 (0.10-6.76)	0.851
Comorbidity				
Musculoskeletal disease	15	1.0 (6.7)	1.15 (0.14-9.39)	0.893
Heart disease	8.1	0.0 (0.0)	0.00 (0.00-Inf)	0.994
Pulmonary disease	8	1.4 (17.5)	3.30 (0.45-23.92)	0.234**
Thyroid disorder	6	0.0 (0.0)	0.00 (0.00-Inf)	0.995
Other possibly impacting disease[3]	9	1.0 (11.1)	2.05 (0.26-15.98)	0.492
Other non-impacting disease[4]	26	0.0 (0.0)	0.00 (0.00-Inf)	0.993

Category	N[1]	PTLDS N (%)	RR (95% CI)	P-value
PTLDS-related symptoms in the months before Lyme borreliosis				
Muscle pain	22.4	2.4 (10.7)	2.24 (0.44-11.39)	0.327
Joint pain	27.6	3.4 (12.3)	3.28 (0.70-15.42)	0.132**
Fatigue	28	1.4 (5.0)	0.75 (0.10-5.95)	0.786
Memory difficulties	11.2	0.0 (0.0)	0.00 (0.00-Inf)	0.993
Concentration difficulties	11.3	0.1 (1.3)	0.00 (0.00-Inf)	0.994
Wording difficulties	15.9	0.2 (1.3)	0.00 (0.00-Inf)	0.995
Symptoms at diagnosis				
N symptoms other than EM[5]			1.17 (0.90-1.52)	0.242**
Multiple EM[6]	5	1.0 (20.0)	3.83 (0.53-27.44)	0.179**
Duration EM			1.01 (0.97-1.05)	0.547
EM diameter[7]			1.05 (0.94-1.16)	0.379
Antibiotic treatment[8]				0.266
Recommended	65.5	3.0 (4.6)	Reference	-
> Recommended	38.5	2.4 (6.2)	1.34 (0.23-7.62)	0.741
< Recommended	4	1.0 (25.0)	5.46 (0.70-42.42)	0.103

** p-value < 0.25, included in multivariable model

N: number, PTLDS: post-treatment Lyme disease syndrome, RR: risk ratio, CI: confidence interval, LB: Lyme borreliosis

[1] The decimal numbers are the result of imputing the variables when missing, with the same value only in part of the repetitive imputations.

[2] One patient living in the Netherlands and one in France, close to the Belgian border, were diagnosed by a GP in Belgium and included in the region Flanders and Wallonia respectively.

[3] Other diagnosis with impact expected on fatigue, widespread pain or cognitive difficulties: sleep apnea, anemia, cancer within past 2 years, hemochromatosis, Ehler-Danlos, depression, Attention Deficit Disorder, hepatitis, Crohn's disease, chronic pelvic pain.

[4] Other diagnosis with no or limited impact expected on fatigue, widespread pain or cognitive difficulties: including gastro-esophageal or intestinal diseases, urinary, eye, skin, liver or gynecological diseases.

[5] Number of symptoms from list: fever, headache, nausea, muscle pain, joint pain, swollen joints, neck pain, night sweats, fatigue, memory problems, difficulties concentrating and problems finding words.

[6] Multiple erythema's reported, not necessarily confirmed with serology.

[7] When EM diameter was < 5 cm (n=10.6), the exact diameter was by default missing and a random value between 2 and 4 cm was imputed for the statistical analysis. When no circle, the average of the longest and shortest diameter was used.

[8] Recommended: 1st (10 days 2x100mg doxycycline), 2nd (14 days 3x500mg amoxicillin) or 3th choice (14 days 2x500mg cefuroxime) following Belgian guidelines [7] or 10 days 1x200mg doxycycline; > Recommended: longer treatment or higher dose; < Recommended: lower dose or shorter treatment.

Appendix H

Alternative scenarios to estimate the proportion of PTLDS

In a first alternative scenario, 5 possible EM cases and 3 possible disseminated/late LB patients were included in addition to the cases in the baseline scenario. A similar proportion of PTLDS was found in EM patients and a small increase in disseminated/late LB patients (Table 6). In a second scenario, insufficiently treated patients, more specifically 4 EM patients who received 100 mg doxycycline a day and one early LNB patient treated during 10 instead of 14 days, were excluded from the baseline scenario. The proportion of PTLDS reduced to 5.2% (95% CI 2.2-12.2) for EM and increased to 22.4% (95% CI 7.3-69.0) for disseminated/late LB (Table 6). In a third alternative scenario using a more sensitive case definition, in which standardized questionnaire scores (covering past 4 weeks) were not included in the criteria for both new or worsened symptoms and daily activities, three additional EM patients fulfilled the PTLDS definition, leading to a proportion of 8.7% (95% CI 4.7-16.4). In two out of these additional EM patients, not having an impact on the EQ-5D-5L question in the past 4 weeks, caused the patient not to fulfill the baseline definition, the third patient also did not have a worsened score on the symptom standardized questionnaires at the correct time points. No additional cases were identified in the disseminated/late LB group and there was only a small difference due to imputation. Finally, in the baseline scenario without multiple imputation, so excluding 3 EM patients and 2 disseminated/late LB patients from the analysis for which the final PTLDS status was missing (censored), the proportions equaled 5.7% (2.6-12.4) in EM patients and 23.1% (8.6-62.3) in disseminated/late LB patients.

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Chapter 6

Lyme Borreliosis in Belgium: a cost-of-illness analysis

Geebelen L, Devleesschauwer B, Lernout T, Tersago K, Van Oyen H, Speybroeck N, Beutels P. Lyme Borreliosis in Belgium: a cost-of-illness analysis.

Ready for submission

Abstract

Background: Lyme borreliosis (LB) is the most common tick-borne disease in Europe and North America, yet its economic burden remains largely unknown. This study aimed to estimate the economic cost associated with the different clinical manifestations of LB in Belgium. **Methods:** An incidence approach and societal perspective were used to estimate the total cost-of-illness for LB in Belgium. Costs were calculated for patients with erythema migrans (EM) or disseminated/late LB, and including patients developing post-treatment Lyme disease syndrome (PTLDS). Direct medical, direct non-medical (transport & paid help) and indirect costs (productivity losses) were included in the analysis. Ambulatory cost data were collected through a prospective cohort study in which patients with LB were followed up during 6 to 24 months after diagnosis (June 2016-March 2020). Hospitalization costs were retrieved from the Minimal Clinical Data registry, a mandatory registry for all Belgian hospitals, linked to the Minimal Financial Data registry. Costs were expressed in 2019 euros. **Results:** The total annual cost associated with clinical manifestations of LB in Belgium was estimated at 5.59 million (95% UI 3.82-7.98). Of these, €3.44 million (95% UI 2.05-5.48) or 62% was related to disseminated/late LB diagnoses and €2.15 million (95% UI 1.30-3.26) to EM. In general, direct medical costs and productivity losses accounted for respectively 49.8% and 46.4% of the total costs, while direct non-medical costs accounted for only 3.8%. The estimated mean costs were €193 per EM patient and €5,148 per disseminated/late LB patient. While patients with PTLDS seemed to have somewhat higher costs compared to patients without PTLDS, the number of patients was too small to have representative estimates. **Conclusions:** We estimate the total annual direct medical costs and indirect non-medical costs associated with LB to exceed €5.5 million per year, almost evenly distributed between EM (40%) and disseminated/late LB (60%). EM costs 26 times less but occurs also 16 times more frequent than disseminated/late LB. The cost burden remains limited by comparison to other infectious diseases due to the relative lower incidence.

Introduction

Lyme borreliosis (LB) is the most common tick-borne disease in Europe. An erythema migrans (EM), a red expanding rash at the site of the tick bite, is one of the first and often the only symptom present. If left untreated the infection can disseminate causing more severe disease such as Lyme neuroborreliosis (LNB), Lyme arthritis (LA), Lyme carditis or acrodermatitis chronica atrophicans (ACA) [1,2]. Even after appropriate antibiotic treatment, persisting non-specific symptoms such as fatigue, widespread musculoskeletal pain or cognitive difficulties have been reported by a subset of patients. This is often referred to as post-treatment Lyme disease syndrome (PTLDS) if symptoms persist for 6 months and are of such severity that they impact daily activities [3]. In Belgium the incidence of LB has been estimated at 103 per 100,000 inhabitants (95% UI 87–120) and a study in 2018 ranked it between high priority diseases, yet, the exact burden remains unknown [4,5]. Cost-of illness studies are an important part of the assessment of the impact of a disease on the healthcare system and society. They allow informing policymakers, formulating cost-effective measures to prevent or control future burden and, together with burden of disease studies, prioritization between diseases. Studies assessing the economic burden of LB are limited, both in North America and Europe [6,7], and, to our knowledge, non-existing for Belgium. The aim of this cost-of-illness study was to assess the economic burden of LB in Belgium, taking into account different clinical manifestation groups and PTLDS.

Methods

The total cost of LB was estimated through an incidence-based costing approach for patients with (i) an EM (including multiple EM) and (ii) disseminated/late LB (LNB, LA, Lyme carditis or ACA), which covers also patients developing PTLDS after (i) or (ii). A societal perspective, which considers costs for the national healthcare insurer, the patients and their employers, was taken. Direct medical costs (ambulatory and hospital

care), direct non-medical costs (transport) and indirect non-medical costs (productivity losses due to sick leave) were included using different data sources. Mean costs were extrapolated to the population level using LB incidence estimates [4] and the number of hospitalizations over multiple years to account for yearly fluctuations.

Direct medical costs

Ambulatory care

The current study is part of a larger project in which a prospective cohort study, "HUMTICK", was set up to collect data on, amongst others, the costs for LB in Belgium. The methodology of this study has been described elsewhere [8]. Briefly, between June 2016 and December 2019, adult patients with (i) an EM or (ii) disseminated/late LB were included in the study through a network of general practitioners and eight Belgian hospitals respectively. The cohorts were followed up and patients developing PTLDS were identified based on the case definition proposed by the Infectious Disease Society of America (IDSA) for which results are published elsewhere (**Chapter 5**). The 108 EM patients and 15 disseminated/late LB patients prospectively recruited for the PTLDS baseline analysis were included in the current cost analysis. Of these, respectively six and three patients fulfilled the complete PTLDS definition (**Chapter 5**). Respectively three and two patients had a missing value for PTLDS and their information was imputed as part of the previous analysis. As either all imputations (n=3) or the majority of the imputations (n=2) equaled 'No PTLDS', these patients were in the current study assigned to the no PTLDS groups.

Data on volumes of resource use, more specifically consultations, medication, but also travel expenses, non-medical paid help, informal care and productivity losses (see further) were collected through patient questionnaires at diagnosis, 1 and 3 months later, and at 6, possibly 12 and 24 months after treatment (depending on the moment of inclusion). A cost diary was provided for patients' personal use. In the current analysis, costs occurring until six months after treatment were included for EM patients

and disseminated/late LB patients and costs occurring until 12 months for patients with PTLDS. Data for T24 were not systematically included as only few were available. The number of LB serological tests performed for EM patients at diagnosis or during the first 2 months after diagnosis, although not required, was estimated from data of the network of sentinel general practices (SGP) which follows up the incidence of EM in Belgium [4]. When ELISA or immunoblot were performed, it was assumed to include both IgM and IgG testing. For disseminated/late LB, the specialists enlisting patients in the cohort study provided this information. For hospitalized patients in the cohort study, costs occurring during these hospitalizations – e.g., part of treatment, laboratory testing – were removed as these are calculated based on separate data (see further). Intravenous treatment was considered to be provided during (day) hospitalization only.

Unit prices in euro for the reference year 2019 were used. For consultations with reimbursed care providers (GP, specialist, physiotherapist, psychologist), these were obtained from the National Institute of Health and Disability Insurance [9] (Appendix A, Table 1). For alternative therapists (osteopath, homeopath, chiropractor) prices are not fixed in Belgium. Based on reports of the Belgian Health Care Knowledge Centre (KCE) [10,11] and the website of the Belgian professional association of osteopathy [12], an amount of €50 per consult was attributed (Appendix A, Table 1). Prices of medication were retrieved from the Belgian Centre for Pharmacotherapeutic Information [13]. For antibiotics, and in line with the Belgian guidelines for economic evaluations [14], the cheapest brand was counted as pharmacists in Belgium are obliged to substitute. For food supplements, not described by the BCPI, prices were obtained from the website of the brand itself or – if not available – online pharmacies. If no brand was given for a product, a random value based on a uniform distribution between the minimum and maximum price of brands available online was drawn. In general, the smallest package size containing the number of tablets used by the patient was counted. If the number of tablets was not specified, the smallest box on the market was counted.

Hospitalizations

Data on the costs of hospitalizations for LB were retrieved from the Minimal Clinical Data registry (MCD) linked to the Minimal Financial Data registry (MFD) for the year 2016. The MCD is a registry mandatory for all Belgian hospitals containing information (such as patient demographics, length of stay, primary and secondary diagnosis, etc.) on both classical hospitalizations (at least one overnight) and day-hospitalizations. Diagnosis coding in the MCD registry is, since 2015, based on the International Statistical Classification of Diseases - 10th revision - Clinical Modification (ICD-10-CM). Hospitalizations were identified based on the primary diagnosis being LB, corresponding to the ICD-10-CM LB codes listed in Table 1.

Table 1. ICD-10-CM codes Lyme Borreliosis, 2016, Belgium

Code	Description
A69.20	Lyme disease unspecified
A69.21	Meningitis due to Lyme disease
A69.22	Other neurologic disorders in Lyme disease
A69.23	Arthritis due to Lyme disease
A69.29	Other conditions associated with Lyme disease
L90.4	Acrodermatitis chronica atrophicans

https://www.health.belgium.be/en/node/27415 (in Dutch)

For the classical hospitalizations (at least one night), hospital specific 100% day prices for the year 2019 were multiplied with the number of inpatient days. Other hospital costs available in the MFD registry for 2016 were converted to the reference year 2019 by applying an inflation of 5.16% (Health index 2016-2019) [15]. The cost of drugs administered during overnight-hospitalizations consists of three components: a lump sum per admission, a lump sum by day (€0.62) and a cost per drug administered. For day hospitalizations (hospital stay without night), there is no lump sum for medication. Day costs, medication and radiopharmaceuticals, include both reimbursed and non-reimbursed costs. In contrast, costs for laboratory diagnostics, medical acts or

implants include reimbursed costs only since information on non-reimbursed costs is not available in the MFD. Costs counted included both LB specific (e.g., antibiotics, LB serology) and non-LB specific costs. Supplements for a single room, costs for food, ambulance, etc. could not be counted as this information was not available in the MCD-MFD data. Such supplements tend to be highly variable between hospitals and depends also on the socioeconomic background of patients [16,17].

Direct non-medical costs

For both travel expenses related to ambulatory care and hospitalizations, volumes reported by the patients in the prospective cohort study were used. The number of kilometers travelled by private transport were multiplied with the Belgian kilometric allowance 2019 (€0.3653). If public transport was used, the ticket price was counted. Paid non-medical help (cleaning lady, babysitter, gardener) was valued using expenditures reported by the patients. Informal care from friends or family was measured but not attributed a monetary value, following Belgian guidelines for economic evaluations [14].

Indirect non-medical costs

The costs of productivity losses (work absenteeism) were estimated using the human capital approach (i.e., without friction period), i.e., the number of hours of sick leave were multiplied with the gross national average hourly labor cost being €40.5 in 2019 [18]. Hours of ambulatory productivity loss (not related to hospitalization) were estimated based on the prospective cohort study responses. In accordance with the Belgian Labour Act, one week of sick leave was counted as 38 hours productivity losses for fulltime work and 19 hours for part-time work. Hours of productivity losses due to hospitalization was calculated based on the mean length of hospital stay for cases < 65 years old in the MCD-MFD data, an employment rate of 65.3% for the Belgian population aged 15-65 years (of which 72% fulltime, 13% 4/5th, 10% halftime and 4.9% other part-time work counted as halftime) [19,20] and, following KCE guidelines,

assuming 18.8 working days per month with 7.6 working hours per day [14]. For day hospitalization, half a day productivity loss was counted (estimation based on the duration of IV treatment administration). For children it was assumed that one parent takes sick leave.

Number of LB cases in Belgium

To extrapolate to the population level, the estimated average ambulatory costs were multiplied with the total number of LB cases in Belgium for each manifestation group based on the average annual incidence reported by Geebelen et al. (2019) for the period 2015–2017 [4]. A total population size of 11,462,024 was applied (Belgium, mid-year 2019) [21]. The PTLDS patients and their costs were included in the cost estimation as part of the group from which they emanated (group (i) or (ii)). For the hospitalizations, the annual mean number of hospital admissions for the period 2016-2018 was used. As there are no specific codes for EM in the ICD-10-CM coding system, and as EM is not expected to cause hospitalization when occurring on its own, the hospitalization costs were assigned to the more serious manifestation group (ii) disseminated/late LB.

Data analysis

All analyses were conducted in R version 3.6.3 [22]. For different cost categories within the ambulatory data and hospital data, uncertainty was propagated performing a non-parametric bootstrap procedure with 10,000 replications to calculate 10,000 bootstrapped means (distributions). The bootstrapped means of ambulatory costs for (i) EM patients and (ii) disseminated/late LB patients, more specifically the bootstrapped means of the total direct medical costs, the direct non-medical (travel expenses, paid non-medical help) and the indirect non-medical costs (productivity losses), were multiplied with the number of cases in Belgium on which uncertainty was propagated using 10,000 Monte Carlo simulations following a uniform distribution defined by the mean minimum and mean maximum incidence estimate for the period

2015–2017 [4]. Bootstrapped means of direct medical and indirect non-medical costs related to (day-)hospitalizations were multiplied with the mean number of stays for the period 2016–2018. The results for the different cost categories (distributions) were further summed up by patient group to calculate total costs for Belgium capturing the uncertainty on the input parameters in the final outputs. All results were summarized by the mean and 95% uncertainty interval (UI) defined as the 2.5th and 97.5th percentile of the bootstrap or uncertainty distribution. Unless specified differently earlier (e.g., medication), missing values were imputed with the median of the observed values in the same group at the same time point or multiple time points combined if n observations < 5. When patients reported a range instead of an exact value, the mean of the range was used.

Results

Direct medical costs

Ambulatory care

The mean ambulatory direct medical cost was estimated at €124 (95% UI 90.3-165)) for EM patients (i) and €516 (95% UI 350-731) for disseminated/late LB patients (ii), including patients developing PTLDS in both groups (Table 2). Estimates for patients without or with PTLDS separately are shown in Table 2. Ambulatory direct medical costs were highest for patients with disseminated/late LB at a mean of €524 (95% UI 327-787) and €478 (95% UI 262-723) for those without and with PTLDS, respectively. Within the EM group, the mean ambulatory direct medical cost was almost twice as high for patients with PTLDS (€214, 95% UI 87.7-382) compared to without PTLDS (€118, 95% UI 85.7-162). The latter was mainly due to a difference in OTC medication (mean difference €65.2) followed by consultations (mean difference €30.1).

Table 2. Estimated mean ambulatory cost per patient, expressed in 2019 euros, for the different manifestation groups of Lyme borreliosis in the prospective cohort study (HUMTICK), 2016–2020, Belgium. Mean of the bootstrap distribution and 95% uncertainty intervals of the mean.

Nb pts in cohort	Unit cost	(i) Erythema migrans			(ii) Disseminated/late LB		
		No PTLDS	PTLDS	All	No PTLDS	PTLDS	All
		102	6	108	12	3	15
Total direct medical costs		**€118 (85.7-162)[1]**	**€214 (87.7-382)[1]**	**€124 (90.3-165)[1]**	**€524 (327-787)**	**€478 (262-723)**	**€516 (350-731)**
Consultations		**€61.5 (41.1-89.1)**	**€91.6 (39.4-146)**	**€63.1 (43.1-89.1)**	**€352 (255-452)**	**€283 (85.3-382)**	**€338 (252-426)**
GP	*€26.3*	€40.6 (34.8-47.6)	€47.9 (30.6-65.7)	€41 (35.3-47.6)	€105.3 (72.2-142)	€105 (0-158)	€105 (73.6-139)
Specialist[2]	*€26.3-60.0*	€11.4 (2.5-23.9)	€15.4 (0-46.5)	€11.6 (3-23.4)	€144 (107-186)	€115 (85.3-140)	€138 (109-173)
Emergency[3]	*€34.9*	€0 (0-0)	€0 (0-0)	€0 (0-0)	€26.2 (14.5-37.8)	€46.4 (0-105)	€30.4 (16.3-44.2)
Alternative[4]	*€50.0*	€5.4 (0.5-13.2)	€16.9 (0-50)	€6.1 (0.9-13.9)	€45.9 (12.5-87.5)	€16.7 (0-50)	€40.1 (13.3-73.2)
Others[5]	*€22.3-60.0*	€4.1 (0-11.7)	€11.3 (0-33.4)	€4.5 (0-12.3)	€30.5 (0-63.8)	€0 (0-0)	€24.3 (0-52.5)
Medication		**€39 (26.4-55.1)**	**€106 (26.6-227)**	**€42.8 (28.9-59.4)**	**€159 (51.9-346)**	**€175 (65.8-341)**	**€163 (66.2-315)**
Antibiotics at T0		€19.5 (17.9-21.1)	€15.1 (12-18.3)	€19.2 (17.7-20.8)	€11.4 (2.9-21.7)	€24.9 (0-41.2)	€14 (5-23.3)
Prescription		€4.2 (0.3-11.2)	€10.7 (0-27.3)	€4.5 (0.6-11.6)	€26.1 (12.9-39.9)	€46.5 (13.4-75.8)	€30.2 (17.4-43.6)
Over the counter		€15.4 (4.6-29.3)	€80.6 (8.9-202)	€19.1 (6.9-34.3)	€122 (18.6-306)	€104 (0-294)	€119 (22.7-273)
LB serology		**€16.3 (13.9-18.7)[6]**	**€16.3 (13.9-18.7)[6]**	**€16.3 (13.9-18.7)[6]**	**€9.8 (0-24.2)[7]**	**€19.8 (0-59.4)[7]**	**€11.6 (0-23.6)[7]**
Other[8]		**€1.5 (0-3.9)**	**€0 (0-0)**	**€1.4 (0-3.7)**	**€3.3 (0-10)**	**€0 (0-0)**	**€2.7 (0-8)**
Indirect non-medical costs							
Ambulatory[9]	*€40.5/hr*	**€62.7 (6-145)**	**€70.5 (0-213)**	**€63.9 (7.1-141)**	**€2,432 (286-5,724)**	**€3,341 (0-5,604)**	**€2,624 (708-5,345)**

Nb: Number; LB: Lyme borreliosis; PTLDS: Post-treatment Lyme disease syndrome; SGP: sentinel general practices; GP: general practitioner; EM: erythema migrans

[1] The bootstrapped means of the total direct medical costs in the cohort study was summed with the bootstrapped means of LB serology in SGP data 2015–2017 and the resulting distribution summarized by the mean and 95% uncertainty interval.
[2] Neurologist, rheumatologist, infectious disease specialist, dermatologist, gastroenterologist, orthopedist, radiologist (consult + forfait by prescription).
[3] Visit and care included only (no medication/performances); price visit without referral by a GP, on a weekday and care provided by a specialist in emergency medicine.
[4] Homeopath, osteopath, chiropractor.
[5] Physiotherapist, psychologist.
[6] Based on SGP data 2015–2017: at diagnosis or in the first 2 months after, ELISA (1st tier test) was performed in 39.7% and Immunoblot (2nd tier test) in 19.0% of EM cases with available information (n=348), no differentiation could be made between patients without or with PTLDS.
[7] Only includes testing performed in patients that were not hospitalized and did not have Lyme neuroborreliosis (n=3), as the latter needs cerebrospinal fluid testing, all tests in these patients and hospitalized patients were expected to be performed during (day-)hospitalization.
[8] Scans: CT-scan, MRI or echo in ambulatory patient.
[9] Productivity losses taking into account part-time work in the cohorts.

Hospitalizations

In 2016, there were 286 classical hospital admissions (for at least one night) for 269 patients in total, with LB as a primary diagnosis. The mean duration of a hospital stay equaled 5.6 days (95% UI 4.8-6.5 days) corresponding to a mean direct medical cost of €4,159 per hospital admission (Table 3). Within the direct medical cost for overnight hospitalizations, hospital day price was the most important cost at a mean of €3,036 (73%), followed by the medical acts (20%), medication (4%) and laboratory tests (2%). LB serology was performed during 79% of stays, at least one antibiotic treatment, possibly for LB, was given during 67% of stays and pain killers during 41% of stays.

Table 3. Hospitalization costs per classical hospitalization (overnight) and day hospitalization, 2016 euros converted to 2019 euros, Belgium.

	Mean unit cost	Cost per hospital stay Mean (95% UI)
Overnight hospital stays (n=286)		
Total direct medical costs		**€4,159 (3,604-4,787)**
100% day cost	**€525**	**€3,036 (2,559-3,593)**
Medication		**€170 (140-213)**
Lump sum per admission	€95.7	€94.7 (92-97.4)
Lump sum per day[1]	€0.62	€3.5 (3-4)
Antibiotics[2]		€13.5 (11.1-16.2)
Pain killers		€1 (0.7-1.4)
Others		€56.8 (28.7-99.7)
Laboratory tests		**€101 (89.1-113)**
LB serology		€13.7 (12-15.3)
Others		€87 (76-98.4)
Medical acts		**€841 (771-916)**
Clinical biology		€228 (204-257)
Permanence, examinations		€204 (189-221)
Medical imaging		€199 (180-219)
Others[3]		€210 (180-242)
Implants[4]		**€5.5 (2.5-9.1)**
Radiopharmaceuticals		**€6.7 (2.9-11.1)**
Indirect non-medical cost (productivity loss)[5]	**€40.5/hr**	**€399 (348-452)**
Day hospital stays (n=613)		
Total direct medical costs		**€50.5 (35.7-69.7)**
Indirect non-medical cost (productivity loss)[4]	**€40.5/hr**	**€29.8**

[1] Lump sum per day: forfait for reimbursed medicines, charged to the patient by day even if such medicines have not been used.
[2] Antibiotics probably related to Lyme borreliosis: ceftriaxone, doxycycline, amoxicillin, clarithromycin, cefuroxime, azithromycin, cefotaxim, ampicillin, cefepime, cefazolin and flucloxacillin.
[3] Including internal medicine, revalidation, surgery, night/weekend supplements and others.
[4] Mainly catheter.
[5] Based on mean bootstrapped days of stay (< 65 years old) * proportion hospitalizations < 65 years old * proportion of < 65 years olds working *proportion working days *7.6 hours * €40.5.

Out of 684 day hospitalizations with LB as a primary diagnosis in 2016, MCD-MFD data were available for 613, comprising 68 unique patients. The mean direct medical cost per day hospitalization equaled €50.5, corresponding to a mean of €454 by patient. The majority of patients (n=57/68) received intravenous antibiotic treatment and medication caused 53% of costs.

Direct non-medical costs

The estimated mean travel expenses related to ambulatory care or hospitalization equaled €5.0 (95% UI 1.0-11.5) in EM patients and €190 (95% UI 92-297) in disseminated/late LB patients. Within the EM group, mean costs were higher in patients with PTLDS (€16.2, 95% UI 0.9-42.1) compared to no PTLDS (€4.4, 95% UI 0.6-10.7). For disseminated/late LB, costs were lower for patients with PTLDS (€97.2, 95% UI 0-219) than no PTLDS (€213, 95% UI 94.4-341), but the number of patients in the disseminated/late LB group and PTLDS groups were low.

Only 6/108 (5.6%) EM patients reported the use of informal care of family or friends compared to 8/15 (53.3%) of disseminated/late LB patients. The mean number of hours of help received was highest for EM patients with PTLDS (18.8, 95% UI 0.33-48.50), followed by disseminated/late LB without PTLDS (12.69, 95% UI 4.7-21.7), disseminated/late LB with PTLDS (2.01, 95% UI 0.0-6.0) and EM patients without PTLDS (1.21, 95% UI 0.0-3.4). Paid non-medical help was only reported by one patient with disseminated/late LB without PTLDS, declaring a cost of €600 (gardener), leading to a mean cost of €40.8 (95% UI 0.0-120) for all disseminated/late LB patients (n=15).

Indirect non-medical costs

The estimated mean costs for productivity losses, not related to hospitalization, by patient group are shown in Table 2. The cost was highest for disseminated/late LB patients with PTLDS, at a mean of €3,341 (95% UI 0-5,604) corresponding to 10.9 working days, followed by disseminated/late LB without PTLDS (€2,432; 95% UI 286-5,724; 7.9 days), EM with PTLDS (€71; 95% UI 0-213; 1.75 hours) and EM without PTLDS (€62.7; 95% UI 6-145; 1.55 hours). The mean cost for productivity losses related to hospitalizations (group ii) was estimated at €399 (95% UI 348-452) per overnight hospital admission and €29.8 per day hospitalization (Table 3).

Total LB cost in Belgium

Table 4 shows the mean number of LB cases, the mean number of hospitalizations and the total cost estimates for the different clinical manifestations of LB for the whole of Belgium per year. The total annual cost, including ambulatory care, hospital care, travel expenses, non-medical help and productivity loss, equaled €5.59 million (95% UI 3.82-7.98). Of these, €3.44 million (95% UI 2.05-5.48) or 62% was related to disseminated/late LB diagnoses and €2.15 million (95% UI 1.30-3.26) or 38.4% to EM. In general, direct medical costs and productivity losses accounted for 49.8% and 46.4% of the total costs respectively, direct non-medical costs accounted for 3.8%. The proportion of costs for productivity losses was higher in disseminated/late LB patients (54.6%) than in EM patients (33.2%). Out of the direct medical costs in all patients, 38.0% was related to hospitalizations or day-hospitalizations, whereas within the disseminated/late LB patient group this was 75.1%. The majority of direct medical costs were reimbursed, namely 75.5%, yet not all non-reimbursed costs for hospitalizations could be counted and the proportion reimbursed depends on the patient's insurance. A division between costs reimbursed by the health insurance system and non-reimbursed costs for the patients themselves is provided in Appendix B (Table B1).

Per patient, the total cost, including ambulatory care, hospital care, travel expenses and productivity losses, corresponded to a mean of €193 for EM patients and €5,148 for disseminated/late LB patients. Regarding the latter, €1,778 per patient was related to hospitalizations (direct medical costs and productivity losses due to regular hospitalization or day-hospitalization).

Table 4. Incidence number of cases and total costs for Lyme borreliosis in Belgium. Total costs and 95% uncertainty interval.

	(i) Erythema migrans	(ii) Disseminated/late LB	Total
Nb cases	**11,168 (9,417-12,954)**	**673 (519-867)**	**11,840 (9,976-13,732)**
Nb overnight- hospitalizations	**0**	**248[1]**	**248**
Nb day-hospitalizations	**0**	**539[2]**	**539**
Direct medical costs	**€1,379,838 (954,446-1,940,745)**	**€1,406,501 (1,210,178-1,645,284)**	**€2,786,338 (2,278,144-3,411,333)**
Ambulatory	€1,379,838 (954,446-1,940,745)	€346,965 (215,580-529,594)	€1,726,803 (1,241,868-2,336,606)
Hospital	€0	€1,032,324 (894,388-1,188,219)	€1,032,324 (894,388-1,188,219)
Day-hospital	€0	€27,211 (19,219-37,548)	€27,211 (19,219-37,548)
Direct non-medical costs	**€56,338 (11,223-130,773)**	**€155,000 (70,394-263,255)**	**€211,338 (108,891-341,204)**
Travel expenses	€56,338 (11,223-130,773)	€127,563 (58,472-215,098)	€183,901 (95,605-298,909)
Informal support (paid)	€0 (0-0)	€27,437 (0-90,998)	€27,437 (0-90,998)
Indirect non-medical costs (productivity loss)	**€713,532 (84,747-1,595,890)**	**€1,880,849 (576,266-3,858,549)**	**€2,594,380 (1,046,331-4,805,398)**
Ambulatory	€713,532 (84,747-1,595,890)	€1,765,836 (464,016-3,739,760)	€2,479,367 (930,220-4,687,748)
Hospital	€0	€98,973 (86,376-112,134)	€98,973 (86,376-112,134)
Day-hospital	€0	€16,040 (16,040-16,040)	€16,040 (16,040-16,040)
Total costs	**€2,149,707 (1,299,782-3,262,206)**	**€3,442,349 (2,048,208-5,480,688)**	**€5,592,056 (3,816,810-7,979,428)**
Total cost per patient	**€193 (121-284)**	**€5,148 (3,091-7,911)**	**€473 (333-654)**

Nb: number; LB: Lyme borreliosis

[1] Mean of 268, 217 and 234 classical hospitalizations (overnight) in 2016, 2017 and 2018 respectively, corrected for the Belgian population mid-year 2019 (n=11,462,024).

[2] Mean of 684, 427 and 489 day-hospitalizations in 2016, 2017 and 2018 respectively, corrected for the Belgian population mid-year 2019 (n=11,462,024). For 2018 the number of chirurgical day-hospitalizations is missing as it was smaller than 5.

Discussion

In order to estimate the economic burden of LB in Belgium, the current study used different data sources (a prospective study and national hospital registers) allowing to include direct medical costs, direct non-medical costs and indirect non-medical costs associated with this multisystem infectious disease.

Based on an incidence approach, societal perspective and 2019 euros, the total annual cost associated with LB in Belgium was estimated at €5.59 million with a 95% UI between €3.82 and €7.98 million. Direct medical costs and productivity losses accounted for the majority of the costs, respectively 49.8% and 46.4% of the total. Despite the much lower incidence of disseminated/late LB manifestations compared to EM, the former accounted for more than half of the total national LB costs per year (62%), because of its high mean cost of €5,148 per patient compared to a mean of €193 per EM patient. While the mean ambulatory direct medical cost was almost twice as high for patients with PTLDS after EM compared to EM patients without PTLDS, patients with PTLDS after disseminated/late LB incurred only higher productivity losses relative to patients without PTLDS after disseminated/late LB but no higher direct medical costs. The latter is unexpected, yet, these results need to be interpreted with caution as the sample sizes for the groups with PTLDS in the prospective cohort study were very small, causing uncertainty to be high. Although the majority of direct medical costs were reimbursed (75.5%), patients still pay €43.9 (EM) or €286 (Disseminated/late) of direct medical costs themselves. In addition, all direct non-medical costs are payed by the patient (i.e., travel expenses: €5 (EM) or €190 (Disseminated/late) and paid help: €50.9 (disseminated/late)), making the cost to the patient still quite high. Furthermore, depending on the employment status and duration of sick leave there can be loss in salary.

For hospitalizations, no differentiation between the groups could be made since there is no specific ICD-10-CM code for EM or PTLDS; all hospital costs were therefore allocated to the disseminated/late LB group. Yet, it needs to be noted that, although no hospital costs are expected due to the mild nature of the symptoms of EM, they may be present in exceptional cases. In our EM cohort (i), one EM patient with PTLDS was hospitalized for one night for additional CSF testing and one EM patient without PTLDS received additional ceftriaxone treatment, yet disseminated LB could not be confirmed in these patients.

Since there are yearly fluctuations in the number of LB cases, this study used mean incidence estimates for a period of three years. Nevertheless, this incidence estimate was probably not representative for the number of LB cases in 2019, as 2019 was a year with an exceptionally low number of tick bites, probably due to extreme dry and warm periods during the summer [23]. It is therefore expected that the total costs for the year 2019 would be lower than the current cost estimates based on the LB incidence 2015–2017, but the latter are expected to be more representative for an average year. As is the case for many healthcare cost data, most costs were skewed with a few patients incurring much higher costs than the majority of patients, causing estimated mean costs to be higher than median costs. For example, for ambulatory productivity loss the median cost equaled €0 in EM patients and €656 for disseminated/late LB patients compared to a mean of €63.9 and €2,624 respectively. Yet, in order to include information on all patients with LB in Belgium, means were extrapolated to the population level and not medians.

Comparison of the current results with studies elsewhere is challenging as there is heterogeneity in the methods used, the patient populations studied and the cost data collected, as well as in the healthcare systems in place in the countries concerned. In Europe, some older studies estimating LB costs from a societal perspective have been performed for Scotland (1999) [24], for LNB patients only in Sweden (2000-2005) [25] and for hospitalized patients in Germany (2008-2011) [26]. Similar to our study, van den

Wijngaard et al. (2017) estimated LB costs for the Netherlands, including direct medical and indirect non-medical costs [27]. For EM, a mean ambulatory cost of €136 was estimated (€122 in 2010 converted to 2019 with Dutch health inflation of 11.7% [28] with the same Purchasing Power Parity for both countries [29]), which is only a little lower than our mean estimate of €193 in EM patients (including 5.9% patients with PTLDS (**Chapter 5**)), taking into account that our study included OTC medication, laboratory testing and travel expenses with a cost of €40, which were not counted in the Dutch study. Van den Wijngaard et al. estimated the mean cost for disseminated LB, including ambulatory and hospital costs, at €6,327 (inflated [28]), which is higher compared to the mean of €5,148 in our study (including 20.9% patients with PTLDS). The costs for patients with persisting symptoms in The Netherlands were estimated as a separate category, with a substantial cost of €6,361per patient (inflated [28]). This is much higher than the additional ambulatory costs found for PTLDS patients in our study, yet for the latter uncertainty was high. Also, patients with persisting symptoms in the Dutch study were defined differently than our PTLDS patients and were included based on a GP diagnosis, so it may be that these were more seriously ill patients; the duration of persisting symptoms in that study equaled 4.6 years. In our study, only costs for one year after diagnosis were included, nevertheless, of those patients with PTLDS and data at T24 (n=6), only one patient reported minor costs at T24 (4 days of paracetamol use). Some studies were also performed in the US, but comparison is even more difficult because of fundamental differences in healthcare system organization, as well as differences in clinical manifestations, with a higher proportional occurrence of LA in the US, representing a higher cost [30–32]. One large retrospective study by Adrion et al. (2015) on health insurance data, estimated costs including patients with PTLDS and reported having any PTLDS-related diagnosis to be associated with a $3,798 higher total healthcare cost [33]. Yet again these patients were defined differently than in our study [33]. Further research on the costs of patients with PTLDS is needed.

In the current study, only costs for patients with clinical LB manifestations, either EM or laboratory confirmed disseminated/late LB, were included (**Chapter 5**). As a consequence, no costs for consultations for a tick bite without LB development were added in the analysis. Yet, some patients might consult a GP with a tick bite due to worries about LB. Based on data available from the SGP network (2015–2017), the yearly number of GP consultations for tick bites can be estimated at 19,422 (95% UI 16,384-22,523), which corresponds to a cost of €510,806 (95% UI 430,901-592,368) (no productivity losses counted). Furthermore, only laboratory tests performed in patients with clinical LB manifestations were included, yet many more LB serological tests are performed each year in Belgium. In the period 2015–2017, in ambulatory care only, an annual mean of 220,270 IgG EIA tests and 11,405 IgG Immunoblots were performed on blood, indicating a low positivity rate (5.18%) for the EIA (first-tier test) as immunoblot is recommended to only be performed when EIA is positive or equivocal. This indicates a potential over-use of these tests without benefits for patient management. Note that these serological tests are not advised for EM diagnosis, as in this early disease stage sensitivity is too low. In addition, tests don't allow to differentiate between active and past infection and are not useful to follow-up antibiotic treatment success [34]. A high economic burden of several millions related to these tests can be expected; the mean annual cost for all LB serological tests (IgG, IgM, EIA, Immunoblot, on blood, on CSF) in 2015–2017 equaled €1,092,801 and in addition a forfeit by analysis by patient (not by test) of €19.84–€30.5, depending on the number of tests performed, applies. More research into these costs and the reasons why these high numbers of tests are carried out is therefore of great importance. Previous studies from Germany and the US have highlighted potential inappropriate and over-usage of LB diagnostic tests [35–37], and consequent antibiotic treatment [35,36]. Also, no costs for prophylaxis treatment were included in the current study.

There are some limitations to the current study. First, for the ambulatory cost calculation, no children were included, hence the same costs are assumed as for adults. Second, as data are based on the incidence estimates 2015–2017 published by Geebelen et al. (2019), limitations mentioned there also apply to the current estimates. As a possible underestimation of the proportion of disseminated/late LB manifestations was suspected, national costs for this group estimated in the current study can also be underestimated. Third, the sample size of the latter group in the prospective cohort study, used for ambulatory cost calculation, was small and uncertainty in the results high. The majority of these patients were also included while hospitalized (n=11/15), which is more than what would be expected based on the number of hospitalized patients in 2016 (n=269) and expected disseminated/late cases by year (n=673). A possible influence of this on the ambulatory cost estimates can't be excluded. A large part of the costs for the disseminated/late LB group was related to hospitalization and were calculated on the much bigger sample size of MCD-MFD data. Fourth, for patients with PTLDS, the sample size was also small and further research into their costs is needed. Fifth, hospitalizations for LB were selected based on ICD-10-coding of LB diagnoses in the MCD-MFD data, which serve for financing and administrative purposes and was not developed for surveillance purposes. Quality of the coding and a possible impact on the cost estimations in this study could unfortunately not be checked. Sixth, while a societal perspective was taken, it was not possible to include all costs that might have occurred. For ambulatory laboratory testing, only LB serology was included. For emergency visits, only the visit and care was counted but no information was available on medical acts (imaging, diagnostics, etc.) during the visit. For hospitalizations, no information on non-reimbursed costs related to laboratory diagnostics or medical acts was available. In addition, no supplements, for example for a one-person bedroom, were included. On the other hand, costs for all types of medication, laboratory testing or performances were included for hospitalizations, including for comorbidities not related to LB (e.g., blood pressure medication). Ceftriaxone treatment was assumed to be given during day-hospitalization only, exceptionally, this might be given at home by

home nurse, yet expected to be rare, and therefore not counted. Seventh, for EM or disseminated/late LB patients, no costs exceeding 6 months post diagnosis were included. After a longer period, it becomes more difficult (also for the patient) to relate specific costs to the disease. Finally, patients with a possible diagnosis (e.g., EM < 5 cm and no tick bite, or LNB without CSF analysis) were not included in the analysis. Although no differences are expected for patients with an EM, dependent on the EM size, differences for disseminated/late LB patients can't be excluded.

Comparison of the results with the cost-of-illness of other infectious diseases is not straightforward as both costs (ambulatory & hospital, direct & indirect) and incidence data need to be taken into account. For several infectious diseases, mean costs per case are lower (e.g., influenza [38,39], rotavirus [40]) or similar (e.g., herpes zoster [41]) compared to LB costs, yet, a higher incidence of these infections in Belgium causes their total national costs to be much higher than LB. For example, for influenza, mean ambulatory direct costs have been estimated at €51-64 per case and hospitalization cost at €2,599, which, with an estimated 400 000 cases [38,39], leads to a national cost of more than €30 million (2011-2012, indirect costs not included). Also for human papilloma virus, where mean costs per case and incidences differ largely by outcome (e.g., cervical cancer vs. anogenital warts), overall national costs are higher than LB [42]. Some other infections, have a lower cost due to a low incidence (e.g., hepatitis A [43]) or due to a low cost per case (e.g., Varicella [41]).

In conclusion, the current study provides an important assessment of the costs related to LB in Belgium which have not been estimated. As EM and disseminated/late LB are estimated to respectively cause about 40% and 60% of the total national costs, both measures to prevent EM, such as prevention of tick bites and fast removal of ticks, as prevention of dissemination of disease, such as informing GPs and the public on EM to improve fast treatment, are essential. While patients with PTLDS seem to have somewhat higher costs compared to patients without PTLDS this needs further research.

Ethics approval and consent to participate

For the prospective cohort study, informed consent was obtained from all study participants, ethical approval was obtained from the "Comité d'Ethique Hospitalo-Facultaire Saint-Luc UCL" (ref.: 2016/13AVR/166) which served as the principal ethical committee for the study and from the ethical committee of each participating hospital (UZ Leuven, Ziekenhuis Oost-Limburg, CHU UCL Namur - site Godinne, CHU de Liège, Clinique Saint-Pierre Ottignies, CHR Namur) and the study was approved by the Commission to Protect the Personal Privacy (Beraadslaging Nr. 16/038, reference: SCSZG/16/144). For the Minimal Clinical Data registry and the Minimal Financial Data registry, all data were linked and pseudonymised by the Technical Cel (NIHDI/Federal Public Service Health) established under articles 155 and 156 of the law of 29 April 1996 on social provisions [44].

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Appendices

Table of Contents

Appendix A

Unit prices ambulatory care (2019)

Consultations

Prices for accredited care providers applicable on 01/01/2019 and reimbursements without increased allowance were counted. For some specialists a higher reimbursement is foreseen for the first consultation when referred to by a GP which was assumed to be the case. For emergency visits, the price without referral by a GP was counted. No additional services were counted unless explicitly reported by the patient (e.g., scan under other expenses) and no additional weekend/evening/night tariffs were counted as this information was not available for all consultations.

Table A1. Consultations

Reimbursed care providers	Total fee	Reimbursed	Patient fee	Source
GP[1]	€26.27	€22.27	€4	[1]
Neurologist	€59.96	€47.96/ *€52.96	€12.00/ *€7.00	[1]
Rheumatologist	€59.05	€47.05/ *€52.05	€12.00/ *€7.00	[1]
Internal medicine/infectious disease specialist	€46.50	€34.50 / *€39.50	€12.00/ *€7.00	[1]
Dermatologist	€33.67	€21.67/ *€26.67	€12.00/ *€7.00	[1]
Cardiologist	€39.38	€27.38/ *€32.38	€12.00/ *€7.00	[1]
Gastro-enterologist	€39.38	€27.38/ *€32.38	€12.00/ *€7.00	[1]
Physical medicine	€29.04	€17.04/ *€22.04	€12.00/ *€7.00	[1]
Radiologist **Consultation +** **Fee prescription (CT/MRI)** **Fee prescription (echo)**	€27.31 + €44.70 €12.59	€19.87 + €44.70 €12.59	€7.44 + €0 €0	[2]
Orthopedic specialist	€26.27	€14.27/ *€19.27	€12.00/ *€7.00	[1]
Ophthalmologist	€26.27	€14.27/ *€19.27	€12.00/ *€7.00	[1]
Physiotherapist	€22.26	€16.37	€5.89	[3]
Psychologist[2]	€60.00	€49.00	€11.00	[4]
Emergency department[3]	€34.90	€13.80	€21.10	[5]
Alternative therapists[4]**:** Homeopath[5], osteopath, chiropractor	€50	€0	€50[6]	[6–8]

[1] Consultation in the consulting room with global medical record.

[2] Psychological session of 60 minutes.

[3] Mean price of a consultation with a specialist in emergency medicine, a specialist in acute medicine, a specialist and a physician with an acute medicine license (all accredited), no referral by GP counted for reimbursement.
[4] No reimbursement counted as it is not standard and depends on the health insurance fund of the patient and the alternative therapist consulted whether or not part of consultations is reimbursed.
[5] A primary consult can be more expensive, for this study it is assumed that the patients have consulted a homeopath before.
[6] The mean of the price range provided by the Belgian professional association of osteopathy in 2019 [8] and the price ranges for a consultation with a homeopath or chiropractor in Belgium reported by De Gendt et al. (2010 & 2011) [6,7] – adding inflation of prices between 2009 and 2019.

Table A2. Antibiotic therapy

	N tablets	Total fee	Reimbursed	Patient fee	Source
Doxycycline 100 mg	10	€6.86	€5.32	€1.54	[9]
Doxycycline 200 mg	10	€8.54	€5.78	€2.76	[9]
Amoxicilline 500 mg	16	€6.76	€5.3	€1.46	[9]
Amoxicilline 500 mg	30	€11.85	€6.82	€5.03	[9]
Amoxicilline 1000 mg	20	€12.73	€7.1	€5.63	[9]
Amoxicilline 1000 mg	24	€13.63	€7.37	€6.26	[9]
Cefuroxime 500 mg	10	€10.63	€6.43	€4.2	[9]
Cefuroxime 500 mg	20	€15.96	€8.1	€7.86	[9]
Amoxi/clav 500/125 mg	16	€10.11	€6.27	€3.84	[9]
Amoxi/clav 500/125 mg	30	€13.65	€7.38	€6.27	[9]
Amoxi/clav 875/125 mg	20	€13.99	€7.48	€6.51	[9]
Tetralysal 300	28	€19.47	€9.21	€10.26	[9]

Ceftriaxone treatment is counted under (day) hospitalizations.

Table A3. Laboratory tests ambulatory care

Type cost	Total fee	Reimbursed	Patient fee	Source
Elisa IgG	€1.95	€1.95	€0	[10]
Elisa IgM	€2.34	€2.34	€0	[10]
Blot IgG	€9.38	€9.38	€0	[10]
Blot IgM	€9.38	€9.38	€0	[10]
Forfait[1]	€19.84 – 36.37	€19.84 –23.41	€0-12.96	[10]

Cerebrospinal fluid testing is counted under (day) hospitalizations.
[1] Amount dependent on number of tests performed.

Table A4. Other ambulatory costs

Type cost	Total fee	Reimbursed	Patient fee	Source
CT-scan knee	€98.65	€96.17	€2.48	[2]
MRI	€52.44	€49.96	€2.48	[2]
Echo lower extremities	€40.13	€37.65	€2.48	[2]

Appendix B

Table B1. Incidence number of cases and total costs for LB in Belgium for the healthcare insurance system or patient. Total costs and 95% uncertainty interval.

	(i) Erythema migrans	(ii) Disseminated/late LB	Total
Nb cases	**11,168 (9,417-12,954)**	**673 (519-867)**	**11,840 (9,976-13,732)**
Nb hospitalizations	**0**	**248**	**248**
Nb day-hospitalizations	**0**	**539**	**539**
Direct medical costs	**Ins.: €889,769 (650,710-1,212,840)** **Pt.: €490,069 (269,825-790,456)**	**Ins.: €1,214,884 (1,069,390-1,385,462)** **Pt.: €191,617 (101,720-333,853)**	**Ins.: €2,104,653 (1,800,383-2,474,597)** **Pt.: €681,685 (425,171-1,021,477)**
Ambulatory	Ins.: €889,769 (650,710-1,212,840) Pt.: €490,069 (269,825-790,456)	Ins.: €184,984 (129,145-256,279) Pt.: €161,981 (72,453-303,738)	Ins.: €1,074,753 (810,758-1,418,809) Pt.: €652,050 (395,997-991,616)
Hospital	€0	Ins.: €1,005,719 (870,347-1,158,271) Pt.: €26,606 (23,041-30,630)	Ins.: €1,005,719 (870,347-1,158,271) Pt.: €26,606 (23,041-30,630)
Day-hospital	€0	Ins.: €24,182 (16,364-34,492) Pt.: €3,029 (2,499-3,941)	Ins.: €24,182 (16,364-34,492) Pt.: €3,029 (2,499-3,941)
Direct medical costs per patient	**Ins.: €79.7 (63.1-102.8)** **Pt.: €43.9 (25.1-68.1)**	**Ins.: €4632 (2615-7430)** **Pt.: €286 (160-476)**	**Ins.: €179 (152-209)** **Pt.: €57.6 (37.5-82.1)**

Nb: Number; Ins.: Insurance; Pt.: Patient

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Chapter 7

No molecular detection of tick-borne pathogens in the blood of patients with erythema migrans in Belgium

Adapted from:

Geebelen L, Lernout T, Tersago K, Terryn S, W. Hovius J, Docters van Leeuwen A, Van Gucht S, Speybroeck N, Sprong H. No molecular detection of tick-borne pathogens in the blood of patients with erythema migrans in Belgium.

Minor revision in Parasites & Vectors

Abstract

Background: Besides the causative agent of Lyme borreliosis, other tick-borne pathogens circulate in the Belgian tick population. However, so far, only a few patients with other tick-borne diseases than Lyme borreliosis have been reported in Belgium. The aim of this study was to investigate the occurrence of other human tick-borne infections and their possible clinical manifestation.

Methods: Patients with fever (> 37.5°C) after a tick bite (< 1 month) or erythema migrans (EM) were included. EDTA-blood was screened for the presence of DNA from *Borrelia burgdorferi* s.l., *Borrelia miyamotoi*, *Anaplasma phagocytophilum*, *Neoehrlichia mikurensis*, spotted fever *Rickettsia's*, *Babesia* spp., *Bartonella* spp., *Spiroplasma ixodetis* and tick-borne encephalitis virus, using multiplex PCR techniques. A questionnaire on, amongst others, demographics and clinical symptoms was filled out.

Results: Over a period of three years, 119 EM patients and 14 patients with fever after a recent tick bite were included. The qPCR of *N. mikurensis* was initially positive for three samples, but could not be confirmed with other PCRs, and repetition of DNA extraction and qPCR was not successful. The qPCR reactions for the other tick-borne pathogens were negative.

Conclusions: In general, only few patients with fever after a tick bite could be identified. Although no tick-borne pathogens were detected, their occurrence cannot be excluded based on a limited number of patients and due to limitations of the detection method used. This study underscores the possibility of false-positive PCR-results and the necessity for the development of new tools for the sensitive and specific detection of emerging tick-borne pathogens.

Introduction

Lyme borreliosis, caused by spirochetes from the *Borrelia burgdorferi* sensu lato (s.l.) complex, is the most common tick-borne disease in Europe and North America [1]. Ixodes ricinus is the main vector of Lyme borreliosis in Europe, and known to transmit several other infectious diseases, including tick-borne encephalitis (TBE), anaplasmosis and babesiosis [2,3]. Some less known pathogens transmitted by these ticks, such as *Borrelia miyamotoi* and *Neoehrlichia mikurensis*, were more recently found to be pathogenic to humans and only a limited number of cases have been described in Europe [4–6]. For others, e.g., *Rickettsia helvetica*, the pathogenicity remains unresolved [7–9]. A Belgian study in 2017 [10] detected several potential pathogens in *I. ricinus* ticks removed from humans with a prevalence of 14% for *B. burgdorferi* s.l.; 1.5% for *Babesia* spp.; 1.8% for *Anaplasma phagocytophilum*; 2.4% for *B. miyamotoi*; 2.8% for *N. mikurensis*; 6.8% for *R. helvetica* and also 13.5% for *Spiroplasma ixodetis* (Supplementary data 1, *available on request to L. Geebelen*). The same study identified *Rickettsia raoultii* in two out of five Dermacentor reticulatus ticks. Co-infections were reported in 3.9% of the screened ticks [10]. In humans, many of these infections might occur asymptomatically or may cause non-characteristic, self-limiting, flu-like symptoms for which patients may not seek medical assistance. However, some could occasionally cause severe disease, especially in immunocompromised patients [3]. In Belgium, no or only few cases of human tick-borne infections other than Lyme borreliosis have been reported [10]. Although tick-borne encephalitis virus (TBEV) was not detected in the screened ticks, the first two human cases, classified as possible and probable autochthonous, have been diagnosed in 2018 [10]. For human granulocytic anaplasmosis, only few confirmed cases have been diagnosed, but every year there are 10 to 20 probable cases based on symptoms and serology and underdiagnoses is suspected due to difficulties in diagnosis and lack of awareness [11,12]. The latter is probably also the case for several other tick-borne pathogens. For human babesiosis for instance, a Belgian study showed the presence of antibodies against three *Babesia*

species in blood from persons with symptoms after a tick bite, yet no human clinical cases have been reported [13]. In addition, although present in ticks, no cases of *B. miyamotoi* disease, caused by a relapsing fever spirochete, or neoehrlichiosis, caused by infections with the intracellular bacterium *N. mikurensis*, have been reported in Belgium [14,15]. The latter, previously called '*Candidatus* N. mikurensis', was only recently cultivated successfully which is why it lost its candidatus status [14]. For the tick-borne spotted fever rickettsiae, such as R. helvetica, R. monacensis or R. raoultii, no confirmed autochthonous infections or cases have been reported in Belgium [10]. Although *Bartonella* spp. DNA can be found in Ixodes ricinus, it remains uncertain whether they are a relevant vector for the transmission of the disease [16,17]. Bartonellosis, mainly Cat-scratch disease caused by *B. henselae*, has been regularly diagnosed in Belgium [18]. Similarly, although found in ticks, the transmission of *Spiroplasma ixodetis*, causing intraocular infection in newborns, by ticks is still unconfirmed [19]. Only a handful of human infections have been described, but so far never in Belgium [20,21].

In general, the incidence and severity of tick-borne infections other than Lyme borreliosis and tick-borne encephalitis are largely unknown and the public health implications remain unclear. Furthermore, concurrent infection with *Anaplasma* spp. or *Babesia* spp. might exacerbate the course of Lyme borreliosis, but information on the impact of other co-infections is lacking [22–24]. Due to the mild non-specific symptomatology, as well as a low awareness among physicians and patients and a lack of routinely available diagnostic tests, it is possible that several tick-borne diseases are underdiagnosed [25]. In addition, many patients don't remember a tick bite, complicating diagnosis.

The aim of this study was to evaluate the presence of tick-borne pathogens other than Borrelia spirochetes using polymerase chain reaction (PCR) techniques in blood from patients with a recent tick bite and fever, a common symptom of these other infections, and in patients with an erythema migrans (EM), the most common clinical

manifestation of Lyme borreliosis. Furthermore, the study aimed to investigate the ability of these pathogens to cause clinical disease, presenting with fever or other symptoms to general practitioners.

Methods

Study design & participant enrollment

Between June 2016 and August 2019, patients with fever (> 37.5°C, reported by the patient or GP), within one month after a tick bite or an EM (including multiple EM) were included in the study by a network of general practitioners (GPs) set up in areas endemic for tick bites and Lyme borreliosis in Belgium. GPs were invited to participate by emails sent through the GP associations from these areas and by personal invitations by post. At the beginning of the study the network consisted of about 50 GPs, which extended during the study period to about 200 GPs from 2018 onwards. After registration, participating GPs received post packages providing information on the study, including guidelines for the diagnosis of an EM [26], blood sampling material and the inclusion questionnaire for both groups. Patients were included prospectively around their diagnosis in order to collect an EDTA-blood sample of 6 ml during the acute phase of illness and before the administration of antibiotic treatment. Patients under 18 years of age and pregnant women were not eligible for participation. Participants were excluded when the geographical location where the tick bite occurred (if known) was not in Belgium, when the EM diameter was < 5 cm and the patient did not recall a tick bite or the delay in appearance was less than 2 days (if known) [26]. The questionnaire consisted of a first part to be filled out together with the GP containing questions on the diagnosis, treatment, comorbidities (including immunocompromising illness or treatments), antibiotic use in the month before the blood sample was taken and symptoms at diagnosis (group fever after tick bite), and a second part on demographics, symptoms at diagnosis (EM patients) and exposure to tick bites, to be completed by the patients themselves.

Sample preparation and molecular testing

The whole blood EDTA samples were sent by the GPs to the Belgian health institute Sciensano, where they were aliquoted and stored at minus 80°C. Aliquots were later send in batch to the Laboratory for Zoonoses and Environmental Microbiology, National Institute for Public Health and Environment (RIVM), in the Netherlands. Whole nucleic acids were extracted from the EDTA-blood samples using robot-extraction (MagNA Pure Compact Extraction Robot; Roche, Basel, Switzerland) on 200 µL of EDTA-plasma (Nucleic Acid Isolation Kit I; Roche) in a diagnostic laboratory setting, following the manufacturer's instructions. To detect potential cross-contamination, negative controls were included in each batch of extractions. All samples were analyzed with different (multiplex) real-time PCRs, based on various genes specific for the following microorganism of interest; *B. burgdorferi* s.l. (two targets, ospA & flab) [27], *B. miyamotoi* (flagellin) [28], *A. phagocytophilum* (msp2) [29,30], *N. mikurensis* (groEL) [31], spotted fever *Rickettsia*'s (gltA) [32], *R. helvetica* (gltA) [33] *Bartonella* spp. (ssrA) [34], *Babesia microti* (18S rRNA) [35], and *Babesia* species from the *Babesia senso stricto* clade (18S rRNA) [36,37]. A qPCR for the detection of *Spiroplasma ixodetis* was newly developed using primers targeting a 170-bp fragment of the RNA polymerase subunit 5'-TGT-TGG-ACC-AAA-CGA-AGT-TG-3' (Spir_rpoB-F), 5'-CCA-ACA-ATT-GGT-GTT-TGG-GG-3' (Spir_rpoB-F), and probe 5'-(Atto425)-GCT-AAC-CGT-GCT-TTA-ATG-GG(BHQ1)-3' [38]. Ticks from a previous study were also tested for *Spiroplasma ixodetis* (results in Supplementary data 1, *available on request to L. Geebelen*) [10]. These qPCRs were carried out on a LightCycler 480 (Roche Diagnostics Nederland B.V, Almere, the Netherlands) in a final volume of 20 µl with iQ multiplex Powermix, 3 µl of sample and 0.2 µM for all primers and different concentrations for probes. Positive plasmid controls and negative water controls were used on every plate tested. The nucleic acid extractions of the EDTA-blood samples were also tested for the presence of TBEV RNA. For the latter, a multiplex reverse transcription real-time PCR was performed as described by Lindblom et al. (2014) [39]. In short, reactions were done in a final volume

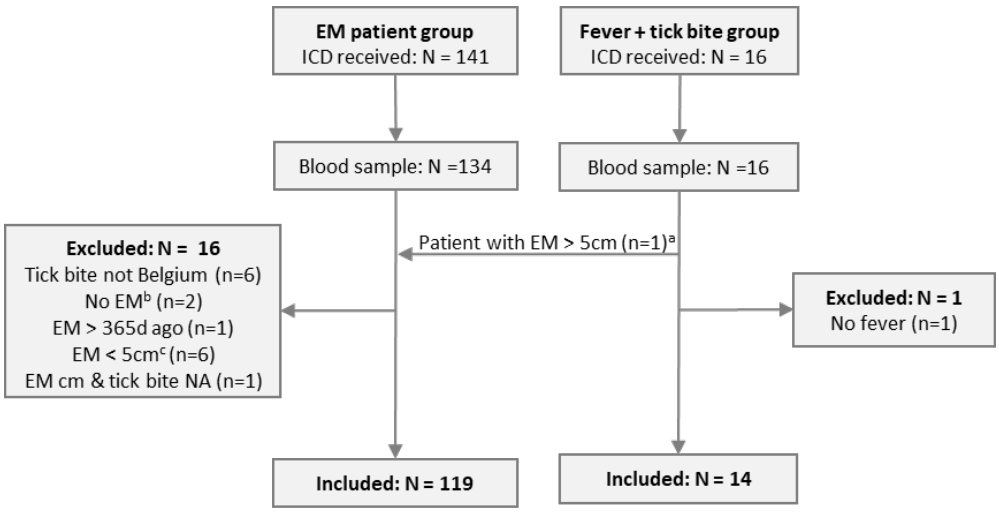
of 20 µl containing TaqMan Fast Virus 1-Step Master Mix (Thermo Fisher scientific, USA) to which 5 µl of sample, 0.2 µM for all primers and 0.2 µM probes were added. An internal control was added to all the samples. The amplification was performed on a Roche LightCycler 480 instrument according to the following program: 20 min reverse transcription step at 50°C followed by denaturation at 95°C for 30 s and 50 cycles of 95°C for 10 s and 60°C for 30 s. Conventional PCRs were performed on all samples that were found positive in the real-time PCR for confirmation on one or more targets followed by Tris-Borate-EDTA-agarose gel-electrophoresis as described [40]. In addition, samples positive in the real-time PCR for *N. mikurensis* were sent by normal mail to another laboratory to be analyzed with a second real-time PCR targeting another fragment of the groEL gene as described previously [41]. More specifically, the SYBR-green PCR set-up was used and either 5 µl or 0.5µl of DNA lysate was added in a final reaction volume of 25 µl. EDTA-blood samples originally positive for *N. mikurensis* were extracted again with the Qiagen DNeasy Blood & Tissue Kit according to the manufacturer's protocol (Qiagen, Venlo, The Netherlands), and tested again with the qPCR for *N. mikurensis* (groEL) [31]. To minimize contamination and false-positive samples, the DNA/RNA extraction, PCR mix reparation, sample addition, and (q)PCR analyses were performed in separated air locked dedicated labs.

Results

Patient characteristics and symptoms

In total, blood samples were collected from 150 patients recruited by the GP network, of which 17 patients were excluded from the study (Figure 1). Out of the 133 included patients, 119 patients had an EM (89.5%) and only 14 patients had fever after a recent tick bite without an EM.

Figure 1. Patient inclusion flowchart



EM: erythema migrans, ICD: informed consent document, d: days, NA: missing

[a] One patient was included in the group of fever after a recent tick bite but moved to the EM group as the GP also reported multiple EM.

[b] EM diagnosis was later disputed by the patients' GP.

[c] EM < 5 cm without recall of tick bite (n=5) or a known delay in appearance of less than 2 days (n=1).

Among these, 62% and 43% were female in the EM patient group and fever after tick bite group respectively. The mean age of all patients equaled 53 years old (range 18-95). The majority of patients (74%) was included in the periods June to August. Of the EM patients with data available (n=118), 64% could remember a tick bite which occurred with a median of 14 days before the diagnosis (range 1-102). Time since first notice of the EM ranged between 0 and 90 days with a median of 7.5 days (n missing = 11). For the patients with fever after a tick bite (for whom remembering the bite was an inclusion criterion), the bites occurred with a median of 20 days before (range 8-60). In 11 EM patients (9%) the EM diameter was smaller than five cm. For those with a larger diameter, the median was 10 cm (5-30cm) (n missing=5). In total, ten GPs reported that their patient had taken an antibiotic in the month before the blood sample was drawn (n missing=2). Of these, six were considered to potentially impact

the PCR result, the other four were taken > 10 days before the tick bite occurred (n=3) or concerned an antibiotic expected to be ineffective (n=1).

In the group of patients with fever (> 37.5°C) after a recent tick bite (n=14), eight measured the fever but five did not (1 missing). Within the EM patient group, five patients (4%) reported fever at inclusion of which three measured (>37.5°C), fever was reported as unknown by 15 patients and missing for one. Other symptoms were reported more often than fever and a higher prevalence was found for each symptom in the group of patients with fever after a recent tick bite compared to the EM group. Of these 14 patients, 100% experienced fatigue, 77% night-sweats, 71% muscle pain (local/general), 71% headache, 57% joint pain, 54% neck pain, 21% swollen joints, 21% cognitive difficulties and 14% nausea occurring since the tick bite. Most common symptoms, reported by EM patients in the 2 weeks before or at diagnosis were fatigue (36%), muscle pain (local/general) (30%), joint pain (26%), headache (25%) and neck pain (21%); other symptoms were reported by less than 20%. Depending on the symptom, data were missing in 0-7% of all patients.

Molecular detection of tick-borne pathogens

Three out of 133 (2.3%) blood samples tested positive in the multiplex qPCR for the presence of *N. mikurensis* DNA with relatively high Ct values (37.2, 37.9 and 38.8) implying low bacteremia. Despite several attempts, the presence of *N. mikurensis* DNA could not be confirmed in the three qPCR-positive samples by conventional PCR, which is less sensitive than the multiplex qPCR (not shown). Therefore, DNA was reextracted from the *Neoehrlichia*-positive blood samples and sent to a laboratory in Norway for confirmation. There, the samples were tested negative. When the initial multiplex qPCR was thereafter repeated, it was also negative. The DNA or RNA of other pathogens was not detected. The three samples initially positive for *N. mikurensis* were from two males and one female EM patients. One patient was a young adult; the two other patients were older than 80 years. Patient characteristics are shown in table 1. None reported

suffering from an immunocompromising disease or using immunosuppressive drugs. None of the patients reported fever, although unknown for one of them.

Table 1. Characteristics of the erythema migrans patients with positive for *N. mikurensis* in initial qPCR

Patient	Age	Gender	Time between tick bite and T0	Symptoms at T0
#1	90+	F	No recall of tick bite (30 days since start EM)	EM (30 cm)
#2	80-84	M	28 days	EM (12 cm)
#3	20-24	M	35 days	EM (8 cm), general muscle pain, neck pain, fatigue, headache, nausea, cognitive difficulties (fever unknown)

T0: diagnosis by general practitioner; EM: erythema migrans (diameter); NA: missing
Note: patient #2 suffered from dementia. The information on subjective symptoms might have been incomplete.

Discussion

In literature, fever has been described as one of the most common symptoms associated with several tick-borne infections other than Lyme borreliosis [3,9]. Nevertheless, this finding is often based on case reports only [9]. Prospective studies investigating the occurrence of these infections in persons bitten by ticks and their ability to cause clinical disease remain rare [9,40,42–44]. By including patients with fever after a recent tick bite, this study aimed at searching for these pathogens in a group of patients expected to be at a higher risk for infection. Yet, despite a broad molecular screening for various pathogens in this study, no tick-borne infections could be detected in the 14 patients included in this group. Possible explanations are that the fever was caused by another infectious agent unrelated to the tick bite or by another tick-borne pathogen not searched for, that the detection methods for the current pathogens were insufficient (see below) or that an antibiotic treatment was taken

before sample collection. All 14 patients experienced other symptoms in addition to fever, of which fatigue, night sweats, muscle pain and headache were the most common.

In the group of patients with an EM, also no tick-borne co-infections were detected. These results are not completely unexpected, given the limited number of patients in the study and limitations of the detection methods used. Prospective studies in other countries, analyzing similar tick-borne pathogens with PCR, also found only few infections. A study in the Netherlands on 626 persons with a tick bite or an EM and a study in Austria on 489 tick bitten persons, both found about 2.6% to be positive for any of the pathogens researched [40,42]. Yet, a Norwegian study in 70 patients with symptoms, mainly EM, found bacterial DNA in 14% of the patients' samples [44]. Even though no tick-borne pathogens were detected in the blood samples in our study, their presence cannot be excluded. PCR might not be sensitive enough to detect cases and additional serological tests could be of value, as was the case in a recent study in the Nordic countries, which found more infections based on seroconversion compared to PCR [43]. In addition, tissue tropism of the pathogens and timing of the collection of the blood sample, have to be taken into account when using PCR as a diagnostic method for all tick-borne diseases [11,45]. *N. mikurensis*, *Babesia* spp., *B. miyamotoi* and *A. phagocytophilum* are expected to be found in the blood, *B. burgdorferi* s.l. on the other hand is not, which explains why none of the EM patients in the study tested positive for the latter [6,11,14,46]. Therefore, we were unable to assess whether the fever in patients with a recent tick bite might have been caused by infection with *B. burgdorferi* s.l. For some pathogens PCR is most sensitive during the acute phase of illness, and the detection period might be short. A Russian study found that *B. miyamotoi* can only be detected by PCR during the first three days of acute disease [47] and *A. phagocytophilum* is expected to be detectible for less than two weeks after disease onset [11]. Although patients with fever after a recent tick bite were expected to be included around this acute phase, for 8 out of 13 patients (1 missing) symptoms started

more than three days before the consultation and for five patients they started two weeks or longer ago. Patients with an EM could have not yet reached or already passed this acute phase since for those remembering their tick bite, it took place with a median of 14 days before inclusion (range 1-102). Finally, antibiotic treatment before sample collection could cause the PCR to be negative. For six patients it was reported that they had taken at least one dose of an antibiotic, potentially impacting the PCR result, before sample collection (2 missing). One of the initially positive patients had taken an antibiotic in the week before the blood draw, but no effect was expected.

Besides possible false negativity, the current study emphasizes possible false positivity of PCR results. Three samples were positive for *N. mikurensis* in the first qPCR performed, yet all three had high Ct values, indicating low levels of *N. mikurensis* DNA in the circulation. Although there were no irregularities or signs of contamination in the first qPCR result (bands correct size, no secondary bands, positive and negative controls correctly analyzed) and previous testing with this qPCR suggested it to be specific [31], the results could not be confirmed by a conventional PCR. As qPCR-reactions are generally more sensitive than conventional PCR and, in our experience, samples with high Ct-values generally remain negative by conventional PCR, this was not completely unexpected. Samples were however further tested with the SYBR-green qPCR from Norway which also did not confirm the positive test results. This second qPCR-protocol uses different primer pairs and yields a longer PCR-product than the first qPCR. The discrepancies between the first and second qPCR might therefore have been due to differences in sensitivity and specificity, caused for example by the reaction conditions or by some critical point mutations that have been described to occur in the GroEl-gene [41]. Yet, to verify this further, the first multiplex qPCR was repeated and this time all three samples were negative. Although some DNA-degradation might have occurred during storage or transport of the DNA samples, as the additional testing was performed 2 years after the initial testing, the results were ultimately regarded as negative. The discrepancies in test results in this study are unsettling and underscore

the need for complementary diagnostic tests, such as serological tools, for *N. mikurensis* to become available in future. The current lack of these tests complicates research on the presence and possible pathogenicity of these bacteria. The recent cultivation of *N. mikurensis* can accelerate the development of these tests [14]. The possibility of false positive results has previously also been emphasized for *B. miyamotoi* [48], highlighting the need for additional independent confirmation of non-routine PCR results for tick-borne pathogens in general.

Detection of *N. mikurensis* DNA by PCR has been reported in several studies. Similar to our results, a study in symptomatic patients in Poland found three patients positive for *N. mikurensis* in PCR (conventional) but reported no infection as these could not be confirmed with sequencing [49]. However, in other studies, such as the prospective studies from the Netherlands, Austria and Norway, mentioned above, *N. mikurensis* was detected in patients, being the most found pathogen with a prevalence of 1.1%, 2.3% and 10% respectively [40,42,44]. The positive patients were asymptomatic or had non-characteristic symptoms such as fever, headache, arthralgia, myalgia and malaise. In addition, *N. mikurensis* has been detected in 2 out of 102 persons bitten by ticks in a study in Sweden [43,50], in five asymptomatic foresters in Poland [51] and in 12 subclinical/asymptomatic immunosuppressed patients in Norway [52]. Clinical cases have been reported in Europe, originating from Germany, Czech Republic, Sweden and Switzerland [4,53,14,54].

In the end, only 14 patients with fever after a recent tick bite could be included in this study over a period of more than three years, whereas the number of patients with an EM was almost 10 times higher. In addition, only five out of 119 EM patients reported fever (yet it was unknown for 15). Different explanations for this low reported prevalence of fever after a tick bite can be considered. The most common tick-borne disease is Lyme borreliosis, for which fever (alone or with an EM) is rare [1,55]. The findings could also suggest that other tick-borne diseases, in which fever are more common, do not often occur in Belgium. Finally, patients with fever probably do not

relate this to a recent tick bite, and if the fever remains mild and is short-lived, do not consult a physician. However, even though these cases do not impact the public health system and only a small burden can be expected for them, more research is needed on whether they develop long-term sequelae and whether they would need treatment. Furthermore, when comparing the number of patients included in both groups, it has to be acknowledged that patients not remembering a tick bite could be included in the EM group (i.e., 36%), but not in the group of fever after a recent tick bite. In addition, it might have been more difficult for GPs to remember to ask a patient about a recent tick bite when fever was present than to include an EM patient. Yet, each year, reminder emails were sent at the beginning and halfway the tick season, emphasizing both patient groups, in order to increase awareness. There was no difference in the work load for the inclusion of patients in both groups for the GPs but for the patients themselves willingness to participate could have been higher for the group with fever after a recent tick bite, as the questionnaire was shorter and there was no follow-up which was the case for EM patients as part of another study [56].

Given the low number of patients with fever after a recent tick bite found in this study, it could be useful for future studies on infections other than Lyme borreliosis to extent inclusion criteria beyond fever, including patients with other flu-like symptoms after a recent tick bite in order to increase the sample size. Furthermore, it might be useful to include patients of which the tick bite occurred longer than one month ago in case *N. mikurensis* detection is aimed for, as it has been suggested in literature that these bacteria persists in the blood for a longer period, even up to months [42,50–52]. It is expected that, in future, more tick-borne pathogens will be discovered due to the increase in molecular methods and availability of next generation sequencing, of which the pathogenicity will have to be investigated [45].

Conclusions

In this study, no tick-borne pathogen DNA/RNA was detected as an infection in blood samples of patients with an EM or fever after a recent tick bite in Belgium and no evidence of clinical disease caused by a tick-borne infection other than Lyme borreliosis was found. However, limitations of the detection method used should be taken into account. Although our study suggests that the occurrence of fever after a tick bite is low, at this moment, it remains impossible to determine the incidence, severity and public health risk of the other tick-borne diseases in Belgium. In order to do so, knowledge on pathogenicity should first be increased, case definitions should be established, and accurate diagnostics, including serology, should be implemented. This study underscores the limitations and the possibilities of false positives by qPCR's and the necessity for the development of new tools for the sensitive and specific detection of emerging tick-borne pathogens.

Ethics approval and consent to participate

Informed consent was obtained from all study participants. Ethical approval was obtained from the "Comité d'Ethique Hospitalo-Facultaire Saint-Luc UCL" (ref.: 2016/13AVR/166) which served as the principal ethical committee for the study. Individual test results of the PCR analysis were not communicated to the GPs or the patients at any time during the study, as was described in the informed consent form. The study has been approved by the Commission to Protect the Personal Privacy (Beraadslaging Nr. 16/038, reference: SCSZG/16/144).

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Chapter 8

Discussion

The main objective of this thesis was to **contribute to the assessment of the burden of LB and other tick-borne diseases in Belgium**.

To achieve this objective, a prospective cohort study (**Chapter 2 and 3**) was set up and different specific studies were conducted:

First, we estimated the incidence of the different clinical manifestations of LB, including disseminated and late LB, in Belgium (**Chapter 4**). Second, the proportion of patients developing PTLDS was estimated in the prospective cohort study. In addition, the occurrence of non-specific symptoms was compared between LB patients and controls (**Chapter 5**). Third, we estimated the costs related to the different manifestations of LB based on different data sources, including the prospective cohort study (**Chapter 6**). Finally, EDTA-blood samples of patients with an EM or fever after a recent tick bite were screened for the presence of several tick-borne pathogens using multiplex PCR techniques (**Chapter 7**).

In this chapter, we will place our findings in a broader perspective, discuss the main strengths and limitations and present recommendations for future research.

8.1. Lyme borreliosis

8.1.1 Incidence estimation

Although several surveillance systems have been in place in Belgium to follow-up LB trends since many years, surveillance data or specific studies thoroughly assessing the occurrence of disseminated or late LB manifestations have been lacking [1–3]. In practice, it is challenging to collect incidence data on these manifestations. As they are more severe and more difficult to diagnose, they are mainly seen by medical specialists which, due to the wide range of clinical symptoms, include infectious disease specialists, dermatologists, neurologists, rheumatologists, pediatricians and cardiologists. In addition, case definitions are based on both the presence of clinical symptoms and laboratory evidence, that includes multiple tests (serum ELISA and Western blot, cerebrospinal fluid analysis, intrathecal antibody production, pleocytosis, etc.), depending on the manifestation [4]. Yet, because of their severity, it is of the utmost importance to also take into account disseminated or late LB manifestations when assessing the burden or costs of LB.

What this thesis adds

This thesis used an alternative method to estimate, for the first time in Belgium, the incidence of disseminated/late LB manifestations, derived from the annual incidence of EM which was estimated through the SGP network at 97.6/100,000 inhabitants (95% UI 82.0–113.0) for the period 2015-2017. Based on the proportional distribution of the other LB manifestations (ratios) to EM in neighboring countries, estimated through a systematic review and meta-analysis, the incidence of disseminated/late LB was subsequently estimated at 5.9/100,000 (95% UI 4.5-7.5) (**Chapter 4**; [5]). This alternative method, combining the ratios with an EM incidence estimate, has not been used elsewhere.

The estimated ratios for the different clinical manifestations could in the future be used by other countries lacking sufficient data on disseminated or late manifestations, if similar *Borrelia* genospecies, geographical and climate characteristics, as well as LB awareness, are expected [5]. For Belgium, the ratios can be applied to newer EM incidence estimates. Nevertheless, it needs to be taken into account that the proportional distribution of manifestations could change over time; as disseminated LB often develops when EM is left untreated, increased awareness on LB by the public and physicians could lead to less disseminated/late LB cases. A decreasing trend in disseminated LB manifestations over the years, possibly related to an increased awareness, was seen in a study on German mandatory notification data, yet needs further confirmation [6].

Furthermore, limitations highlighted in **chapter 4**, including a possible under- and overestimation of the proportion of disseminated cases, need to be taken into account. One of these concerned the decision to include only studies based on confirmed disseminated/late LB cases in the systematic review to estimate the ratios. It was expected that this could have led to an underestimation of these LB manifestations other than EM; as several laboratory results are required, chances are higher that there is missing information causing them to be classified as probable or possible cases (**Chapter 4**; [5]). The prospective cohort study showed that this can indeed be the case; in addition to the 15 patients with confirmed disseminated/late LB included in the study there were 3 possible cases with suspected LNB, for which no CSF analysis was performed (not desired by patient, n=2) or was performed only after treatment (n=1) (**Chapter 5**). However, the extent of this possible underestimation cannot be determined based on this study.

This thesis also showed that the EM incidence estimated for 2015-2017 was comparable to the EM incidence in 2008-2009 [2]. Similarly, other data sources did not indicate a significant increase of LB in the Belgian population over the past decade [1,3,7].

Future perspectives

Further close surveillance of LB incidence trends over time is essential as changes might occur due to climate change but also due to behavioral changes of the population and e.g., policies towards protection of wildlife and creation of green spaces [8]. At date, it cannot be determined if climate change will lead to an increased or decreased LB incidence in Belgium. Warmer winters could lead to increased tick activity (longer tick season), yet, increasing long dry periods and extreme warm temperatures in summer could reduce tick activity and survival, hence LB risk [9,10]. In addition, several other factors impacted by climate change, such as land use and host availability, are at play [9,10]. As environmental factors also cause annual fluctuations in the number of tick bites and consequently LB incidence, a continuous surveillance is important [11,12]. Hence, we believe that the EM incidence surveillance through the SGP network, which was continuously implemented between 2015 and 2020 but stopped in 2021, should be resumed from 2022 onwards. These yearly fluctuations were also shown in the current study, where a higher EM incidence was found for the year 2016 (106.1 cases/100,000, 95% UI 90.1–122.2) [5]. If only data for that specific year would have been available, this could have led to an erroneous suspicion of an increase of LB incidence over time. Yet, in 2017 the incidence was again lower. Similarly, the number of tick bites registered by the Belgian population through www.tekenet.be fluctuates yearly with fewer tick bites registered in 2017, and even less in 2018 and 2019, probably due to dry and warm periods in the summer [11–13]. In 2020, again more tick bites were registered, yet preliminary analysis of the data of the SGP network indicates a lower incidence of EM compared to previous years, which suggests underreporting or underdiagnoses due to the impact of the COVID-19 epidemic on the healthcare system and reporting in 2020 [14]. This highlights the importance of active cooperation of GPs in this network and the benefits of having multiple surveillance systems for LB. As there are important geographical differences in LB incidence, it is all the more important that the network equally covers high and low incidence areas, hence is representative for

the Belgian population in all regions and provinces. As the number of participating GPs has been decreasing in the latter years, there has been a lower coverage in certain areas which should be improved in the future (e.g., Limburg, Namur). In addition to these known limitations of surveillance systems (participation rate, geographical representativeness), other influencing factors such as awareness of GPs and patients, can contribute to differences in notification of EM by the SGP network in the different regions, complicating interpretation of data.

Ideally, in addition to these currently existing surveillance systems in Belgium, data on the occurrence of disseminated or late LB manifestations should be routinely collected. To allow better comparison between countries and regions and to allow assessment of disease burden and cost of illness of LB in Europe, in 2018, the European Center for Disease Prevention and Control (ECDC) has introduced systematic surveillance of LNB for its member states [15,16]. LNB was chosen for two main reasons; 1) it is one of the most frequent disseminated manifestations but still not too frequent (in opposition to EM) making the registration feasible for the member states; 2) it is the manifestation for which a specific case definition could be proposed, allowing comparison between countries [16,17]. Nevertheless, implementation of such surveillance remains challenging and only 14 out of 30 countries have reported data for 2018, 2019 and/or 2020 (ECDC EVD network meeting 2021). For Belgium, a first feasibility study has been performed in Flanders which concluded that an exhaustive LNB surveillance, as proposed by the ECDC, is not feasible based on existing surveillance systems (such as laboratory surveillance) as data necessary for a confirmed case (clinical data + pleocytosis + CSF intrathecal production) come from different laboratories and services, hence are not easily available. In addition, the sentinel laboratory network is not exhaustive, and clinical experience revealed that a CSF sample is not always taken (see above). However, set up of a sentinel network of neurologists could be feasible and would allow (retrospective) reporting of LNB cases (including confirmed, probable and possible cases). Although not being exhaustive,

such a surveillance would allow to follow trends. Ideally, pediatricians and infectious disease specialists should also be involved in the network as they diagnose additional LNB cases. Nevertheless, as LNB only represents a minority of LB cases, its surveillance cannot serve as a replacement of the other surveillance systems existing and EM surveillance through the SGP network remains essential. Availability of surveillance data for both LNB and EM, would allow to follow-up and compare with other countries, both LB trends over time and changes in the distribution of the different clinical manifestations. In combination with the ratios estimated in this thesis, the incidences of the other manifestations (e.g., LA) could be estimated [5]. A resolution of the European Parliament on LB, approved in November 2018, requests mandatory notification of LB in all affected Member States [18]. Nevertheless, as described by van den Wijngaard et al. (2017), mandatory notification of all LB manifestations is expected to be subject to underreporting as it depends on the motivation of all healthcare professionals and, especially in high-incidence countries, implicates a high workload [6,16,19]. When a sentinel network for the surveillance of LNB is set-up in Belgium, it can serve as a learning experience for other European countries, which then can, possibly, also use it as an alternative for mandatory notification.

8.1.2 Post-treatment Lyme disease syndrome

Although the incidence estimates in **chapter 4** provide essential information on the occurrence of the different clinical manifestations of LB, data on persisting non-specific symptoms after LB have been lacking in Belgium. These symptoms have been a concern since many years, in Belgium, but also in other European countries and north America, with ongoing debate regarding their prevalence, cause and treatment [20–22]. **Chapter 5** of this thesis contributes to an improved understanding of the occurrence of these symptoms in LB patients after treatment.

What this thesis adds

An important added value of the prospective cohort study was the inclusion of a control group. Whereas fatigue, pain and cognitive difficulties have been reported in literature as frequent symptoms related to PTLDS [23–25], only the former two were significantly more often reported by the LB patient cohorts compared to the control group in our study (**Chapter 5**). For the occurrence of at least one PTLDS-related symptom combined with an impact on daily activities, a significantly higher risk compared to the control group was only found for disseminated/late LB patients, at both 6 and 12 months follow-up.

Most importantly, the cohort study demonstrated the occurrence of PTLDS in both patients with an EM (n=108) and patients with disseminated/late LB (n=15), with an estimated proportion of respectively 5.9% (95% CI 2.7-12.9) and 20.9% (95% CI 6.8-64.4). For these estimates, strict criteria with regard to new or worsened symptoms, impact on daily activities and a time period for the symptoms to be present were applied, following the case definition published by the IDSA in 2006, which, to our knowledge, has not been done in this way before [23]. Although these results suggest an increased risk for patients with disseminated/late LB (RR 3.53, 95% CI 0.98-12.68, p=0.053), no significant risk factors could be identified in this thesis, which may be explained by small sample sizes, the main limitation of the current work (**Chapter 5**).

Similar to previous studies, our study showed an important background prevalence of non-specific symptoms. Yet, by assessing new or worsened symptoms, our study, in part, cancelled out this background “noise” which allows better identification of LB-associated symptoms, as described by Strle K. and Strle F. (2020) [26]. Exclusion of symptoms explained by other causes also contributes to this identification of LB-associated symptoms. However, depending on the sample size, individual patient assessment can be challenging in research settings. In the current study, other causes were assessed for the PTLDS estimation but not for the comparison between patients

and controls (**Chapter 5**). Ideally, patients are examined at follow-up by a physician but this is time consuming and costly. This high background prevalence also highlights the importance of the inclusion of a control group, which has been the case in only few studies performed up to now [27–32]. As shown in this study, some differences might exist in the health of LB patients before disease and the health of controls before participation. Whether these differences were caused by recall bias or are true differences cannot be concluded based on current data. Many studies only compare groups at diagnosis or follow-up, but do not have information on health status before LB or participation [27–29,32]. One of these studies found that the overall prevalence of nonspecific symptoms was higher in controls than patients at diagnosis/enrollment, yet not at follow-up, hence data collection on previous health would have been informative [29]. This again highlights the importance of looking at new/worsened symptoms instead of overall prevalences.

In general, inclusion of a comparable control group in epidemiological studies is challenging. Ideally, controls have similar characteristics compared to the patient group, except for the disease to have occurred. Yet, in practice differences often exist. By requesting patients to include controls from their environment, we hoped to have an easy, not time-consuming, inclusion method with a high response rate, due to a greater interest in the subject from family and friends of patients affected by the disease compared to random people. In addition, this method allowed taking into account age (± 5 years) and gender in order to obtain frequency matched cohorts. Recruting controls by the GPs was not used, to avoid the selection of a control group with more comorbidities and to limit their work load. Inclusion of controls by having patients select persons in their environment (spouses, family or friends) has previously been used in other European prospective studies on post-treatment symptoms after LB [27-29]. In practice, many patients did not include a control person in our study and the inclusion method had to be adapted over time (**Chapter 3**). How patients chose the control persons included (e.g., what their relation was) and why some had difficulties

with including controls, is unfortunately not known. More research on existing patterns in control selection and its consequences would be interesting. If it is true that patients bitten by ticks are healthier than the general population, it is of interest to include a control group of persons bitten by a tick who did not develop LB. The latter has been done in a recent large study from the Netherlands, yet they did not find differences in the prevalence of persistent symptoms between the tick bitten group and a second general population control group [32]. In general, inclusion of multiple control groups, as done in the latter study, can be of value [32].

Future perspectives

The observations in the current study call for further research into these non-specific symptoms after LB. While some risk factors have previously been described (dissemination, delay in treatment, symptoms at diagnosis, older age, female gender, etc.), there is still uncertainty regarding them and studies with large sample sizes are needed [25,29,33–39]. Also for the cause and underlying mechanisms of these symptoms, several hypotheses have been proposed but thus far, there has not been any consensus [40]. Filling these knowledge gaps would allow early identification of patients at increased risk, better diagnosis of patients with PTLDS and would contribute to an improved patient management and possibly the discovery of a treatment. Additional knowledge on how best to inform patients with LB on these symptoms and how to communicate with patients with PTLDS, would be of great value for clinical practice. A recent study on prolonged antibiotic treatment in patients with persisting symptoms attributed to LB, showed that the patients' expectancies regarding symptom improvement were predictive for a beneficial symptom course [41]. The impact of such expectancies at the initial diagnosis of LB on the further symptom course has, to our knowledge, not yet been investigated.

If PTLDS is a post-infectious syndrome, as seen with other infectious diseases, upcoming large-scale research into long-COVID might provide new insights into these syndromes

and their possible treatments [42]. Nevertheless, it is not known if there is a common underlying mechanism. In general, more comparative studies with other syndromes including these post-infectious syndromes but also fibromyalgia and chronic fatigue syndrome, would be of added value. To date, only a few studies have been conducted in which both similarities and differences were reported [43–46].

The current thesis also highlighted the impact of the use of different case definitions (e.g., with or without impact on daily activities) and as such stressed the need for a single definition to be used in future studies (**Chapter 5**). In addition, there is a need for the development and validation of standardized methods that are easily applicable in practice to assess the definition. These implementations would allow easier interpretation of and comparison between study results from different countries. One of the few PTLDS case definitions proposed up to now, is the one published in the clinical LB guidelines of the IDSA in 2006 used in this thesis [23]. Yet, the definition was proposed for research only and to avoid its use in a clinical setting, it has not been included in the newer clinical guidelines published by the IDSA in 2021 (personal communication) [47]. Nevertheless, the 2006 definition has been used (sometimes partially) and referred to by several studies [24,30,31,34,37,39,48–50], including a recent large prospective study on more than 1,000 LB patients in the Netherlands [32,51]. Aucott et al. (2013) also assessed it as useful for identification of patients with PTLDS, yet highlighted the need for further refinement and improvement, on which we can agree as it is still allowing open interpretation [48]. Up to now, some efforts have been made to develop such standardized methods, but no generally accepted method exists [30,31,48,50,52]. Without a uniform definition, it is not only challenging to conduct and compare research, but also impossible for clinicians to diagnose patients with PTLDS. Additional future studies towards identification of biological markers to diagnose PTLDS are also of the utmost importance. To reach sufficiently large sample sizes, European research collaborations should be encouraged.

Our cohort study only included patients in which LB diagnosis was confirmed by a GP or medical specialist, using strict case definitions which include the presence of objective clinical manifestations of LB (**Chapter 5**, Appendix A) and all patients received an antibiotic treatment at diagnosis [4,17,53]. As such, the current work is lacking an evaluation of the broader group of patients that often has been referred to as having "chronic Lyme disease", including patients with or without evidence of *B. burgdorferi* infection [20]. As a consequence, this thesis cannot answer all existing questions and expectations of politicians or patient organizations regarding non-specific symptoms after LB. Further research into these other patient categories, for which it is known that they suffer extensively, not only from their symptoms but also because of a lack of understanding, should be performed [22]. Data on the costs and health of patients recruited through a LB patient organization in Belgium have been collected by UGent and Time for Lyme but results are not yet published [54].

8.1.3 Costs and burden

The results of **chapter 4** and **chapter 5** are crucial elements when one wants to estimate the national costs or burden related to all LB cases in Belgium. Such economic and health burden estimates are important to further assess the impact of this disease on the Belgian population and healthcare system which in the end allows to inform public health decision making.

What this thesis adds

This thesis provided a first important estimate of the costs related to LB in Belgium with a substantial national cost estimated at €5.59 million (95% UI 3.82-7.98) (**Chapter 6**). In addition, the study showed that a large part (38%) of the costs was related to EM (representing about 95% of the manifestations) but the majority (62%) were related to disseminated/late LB despite its much lower incidence. Unfortunately, the small sample sizes of the groups of patients developing PTLDS did not allow to draw strong conclusions on the specific costs for this manifestation separately and further research

would be of value. In general, the cost burden remains limited by comparison to other infectious diseases such as influenza, rotavirus and herpes zoster due to the relative lower incidence of LB [55–58].

According to the SGP data 2015-2017, laboratory testing was performed in almost 40% of EM patients, yet this is not routinely advised due to the low sensitivity for diagnosis in this early disease stage and the poor utility of antibody tests for the follow-up of treatment. Performing these tests does not only imply redundant costs but can also lead to confusion for the patients on their diagnosis. In addition, within the prospective cohort study, 35.6% of EM patients in the PTLDS analysis received a treatment in a higher dose or for a longer period than advised in the Belgian guidelines (**Chapter 5**).

Future perspectives

As the national costs were largely related to both EM and disseminated/late LB, measures to prevent and control all manifestations remain important. These include the prevention of tick bites and fast removal of attached ticks (prevent LB) but also, timely treatment of EM (prevent dissemination). The current cost estimates can be used in future cost-effectiveness analyses, e.g., when a LB vaccine becomes available on the market.

Nevertheless, the limitations of the current study should be taken into account (**Chapter 6**). These include ICD-10 coding being used to select hospital admissions and to calculate related costs from the MCD-MFD databases. Some European studies have shown that the quality of the ICD-coding for LB has been questionable in the past [59,60]. However, differences in the coding system between countries exist and Belgium has the advantage of having a LNB specific code which could improve data quality, yet, future validation of the Belgian system would be of great value.

Furthermore, future studies linking different datasets including diagnostic codes (e.g., based on GP reports) on the one hand and cost data, e.g. insurance data, on the other hand, would be of interest for the further validation of the current cost estimates. Such studies allow to select, within the same cost database, a group of patients with LB and compare their cumulative costs to a group of control persons without a LB diagnosis while controlling for other variables (such as age, gender, region). The advantage is that this allows costs incurred over a much longer period of time to be taken into account, hence, including costs possibly related to PTLDS over the longer term. Such a study, based on insurance data for LB, has previously been conducted in the United States [61].

What is lacking in this thesis is a thorough analysis of costs related to laboratory testing for Lyme, in patients other than those eventually diagnosed with LB manifestations. Since a high total number of LB tests are performed yearly (>400,000 ELISA tests and >20,000 immunoblots in 2015-2017), a substantial cost is expected and further research is needed. As these tests do not differentiate between past and current infection, inappropriate use can also lead to inappropriate prescription of antibiotic treatments. Although costs related to this are expected to be limited, they imply a risk on side effects and potential development of resistant bacteria. As indicated by the 40% laboratory testing in EM patients, guidelines are not always followed, therefore re-distribution of the guidelines among GPs would be useful.

Furthermore, this thesis has been limited by the fact that the economic burden of LB was quantified, but in the end, not the health burden, expressed in Disability-Adjusted Life Years (DALYs) as was initially foreseen (**Chapter 2**). The latter has been calculated for the Netherlands by van den Wijngaard et al. (2015) who concluded that the majority of the disease burden related to LB is caused by the small group of patients with persisting symptoms attributed to LB (by the patient and GP) [62]. In addition, some other studies have reported non-specific persisting symptoms after LB to greatly impact quality of life [63,64]. Yet again, inclusion of different study populations and the

lack of use of one specific case definition complicates comparison and interpretation. Further analysis of data collected on the health burden of LB in the current prospective cohort study will be performed, yet again for patients with PTLDS, sample size will be small and further research is encouraged.

8.2. Other tick-borne infections

Although reported far less frequently than LB, infections with other *I. ricinus*-borne pathogens may cause human disease in Belgium as well. With the exception of TBE, a lot of uncertainty regarding the pathogenicity, symptomatology and epidemiology remains for the other tick-borne infections. A study investigating the occurrence and possible clinical manifestations of these other tick-borne infections was performed in the current thesis (**Chapter 7**).

What this thesis adds

In the end, no infections were detected in the blood of 119 patients with an EM and 14 patients with fever after a recent tick bite when using multiplex PCR techniques. Nevertheless, valuable lessons can be drawn from the results presented in **Chapter 7**, not only for future surveillance and research in Belgium, but also for other countries currently considering to perform research on these other tick-borne infections. Our study suggests that the occurrence of fever after a tick bite is low. This may indicate that tick-borne diseases other than LB do not often occur in Belgium. However, it is also possible that patients and GPs do not relate fever to a recent tick bite, that patients don't visit a GP when only mild fever occurs or that they don't remember the tick bite. The difficulty to include patients with fever after a recent tick bite in this thesis should be taken into account when setting up similar future studies. This can be done for example by considering broader inclusion criteria, such as flu-like symptoms after a tick bite but not necessarily fever.

Furthermore, the study underscored important limitations of qPCR tests, including the possibility of false positive results in addition to known limitations such as a lack of sensitivity. As a PCR test on a blood sample is only sensitive for some pathogens, due to tissue tropism, and only during a short period of time after infection, a negative test result can also not rule out infection. Therefore, collection of serum samples would have been an added value for the current study. However, specificity and/or sensitivity of available serological tests can be low during the early phase of infection and consecutive samples would have been necessary to detect seroconversion or a rise in antibody titers, hence acute infection [65,66]. In addition, serological tests are not available for all pathogens [8].

Future perspectives

Although no tick-borne pathogens were detected, their occurrence cannot be excluded based on the limited number of patients in this thesis and due to the methodology used. The study indicates the necessity for the development of new tools for the sensitive and specific detection of emerging tick-borne pathogens. Even if a pathogen is expected to mainly cause asymptomatic infections or only mild flu-like symptoms, and is therefore expected to have only a minor impact on the economic and health burden, availability of accurate tests has added value. Without such tests it is currently very difficult or even impossible to determine whether or not a potential pathogen is the causative agent of an infectious disease. In addition to more traditional clinical and microbiological tools, epidemiological and serological approaches are nowadays necessary to provide evidence for disease causality [67]. This is even further complicated by the possible occurrence of co-infections with multiple tick-borne pathogens, impacting the clinical presentation.

At the moment serological tests are commercially available for some pathogens (TBEV, *A. phagocytophilum*, *Babesia microti* and spotted fever *Rickettsia's*), yet for other pathogens they are still in an experimental phase (e.g., *B. miyamotoi*, other

Babesia spp.) or not yet existing in general (*N. mikurensis*) and further development is needed [68–70]. In addition, several available serological tests are still based on antigens from non-European strains (*A. phagocytophilum*, *Babesia microti*) and the detection of European strains is therefore based on detection through cross-reactivity [8]. The latter is also the case for spotted fever *Rickettsia's* where serology is based on *R. conorii* [8,71]. PCR tests are available for the pathogens described in this thesis, yet, as discussed above, independent confirmation is important and sensitivity might be low. In Belgium, PCR tests for relapsing Borrelia are now available which also detects *B. miyamotoi* [72] but they are currently still lacking for *N. mikurensis*. Microscopic examination, which can be used for *Babesia* spp. and *A. phagocytophilum*, and cultivation have been shown to be of limited use for other tick-borne pathogens [8,23,73]. To overcome currently existing limitations, new methodologies which aim to have high sensitivity and specificity, are currently being developed (e.g., urinary peptides detection [74,75], peptide arrays [76], T-cell tests [77]) and should be further developed in future.

It can be expected that, with increasing awareness and availability of diagnostic methods, more other tick-borne pathogens and infections will be identified in the coming years. Since the identification of autochthonous cases of TBE in 2016 in the Netherlands, the number of tests requested in Belgium has also increased and the first autochthonous cases have been reported in Belgium in recent years [78]. Nevertheless, TBEV antibodies have been found in animals in Belgium for a longer time, indicating previous circulation of TBEV [79]. As TBE can be severe, further surveillance in ticks, animals and humans is important.

In addition, efforts should be made to further increase awareness among clinicians on the risk of autochthonous TBE infections and to encourage them to take TBE into account as a differential diagnosis. This has been done through a news bulletin (monthly) on infectious diseases in November 2018 (Belgium) [80] and through a letter to physicians at the start of the tick season in 2019 (Flanders), and should be repeated

in future. On the other hand, for several of the other tick-borne diseases, knowledge on pathogenicity should first be increased, case definitions should be formulated, and accurate laboratory tests should be developed and made routinely available.

In order to evaluate the risk of human exposure to tick-borne pathogens in Belgium, studies on the prevalence of pathogens in ticks biting humans, as previously performed in 2017 and currently repeated in 2021, are of great importance [81]. Nevertheless, it needs to be taken into account that not all organisms found in ticks can be transmitted or cause disease in humans [67]. Where the latter is the case, further research into the occurrence, clinical disease and burden of these infections is needed. Therefore, in the future, studies like the one conducted in this thesis, yet with the necessary modifications to include more patients with symptoms after a tick bite, when more and better diagnostic tests are available, are of great importance.

8.3. General strengths and limitations of the study

Strengths:

- Prospective study design, following up LB patients from the time of diagnosis onwards, minimizing recall bias.
- Multidisciplinary approach with GPs and different types of medical specialists including patients with an EM and patients with disseminated/late LB.
- Collecting data on different topics allowing to address multiple relevant research questions in one large study.
- Inclusion and follow-up of a non-LB control group allowing to take into account the background prevalence of non-specific symptoms.
- Assessment of new or worsened symptoms.
- Detailed assessment of the different components of the PTLDS definition.
- Use of standardized questionnaires.
- Data on health before LB (patients) or participation (controls).

Limitations:

- Data collection in the SGP network and prospective cohort study is based on voluntary participation of GPs who are experiencing an important increase in administrative workload leaving less time to participate to studies.
- Both under- and overestimation of EM cases possible by SGP network, no further validation of reported cases.
- Confirmed disseminated/late LB possibly underestimated due to multiple criteria in case definition and possibility of missing data.
- Small sample size in the prospective cohort study: mainly for patients with disseminated/late LB, patients with fever after a recent tick bite and cases that developed PTLDS, leading to high uncertainty and low power for risk factor analysis, not allowing inclusion of confounders in the analysis.
- Data for inclusion not received for all patients that agreed to participate at GP consultation.
- Not all patients followed-up at T12, only few at T24.
- No clinical assessment by GP or study investigator at follow-up, also no GP questionnaires at follow-up.
- Health before LB (patients) and before participation (controls) somewhat different.
- Impossible to account for possible psychological effect of receiving a disease diagnosis.
- Although not the goal of this study, the study cannot answer several existing questions of patient organizations and their patients which may be disappointing.
- LB cost estimations related to hospitalizations are based on ICD-10 coding which should in the future be validated for Belgium.
- Limitations of laboratory testing, namely qPCR testing on blood samples, performed in the study on other tick-borne diseases.

8.4. Summary recommendations for future surveillance and research

Belgium:

- **Reimplementation of continuous surveillance of EM through the SGP network.** The network should be representative for all regions.
- **Implementation of surveillance of disseminated LB, more specifically LNB (sentinel network).** This would allow to validate the incidence estimates in the current study, follow-up disease trends over time and together with the EM data, follow-up distributions of the different clinical manifestations of LB.
- **Cost analysis of all laboratory tests performed for LB.** Increased knowledge on reasons for test requests and possible over-use is needed.
- **Further prevention of LB is important to limit costs and burden related to the disease.** These should focus both on prevention of tick bites, early LB (EM) and disseminated/late LB.
- **Estimate the burden of disease of LB in Belgium**, expressed in DALYs
- **Repeat the study on other tick-borne pathogens when more tests are available.** Broader inclusion criteria should be used. **Further follow-up of the prevalence of pathogens in ticks**.
- **Distribution of LB guidelines to health professionals.** Further increase in knowledge on diagnosis and treatment is needed. Update version when necessary.

Europe:

For future research, international collaborations allowing to include a large number of patients would be of great value.

- **Formulate a case definition for PTLDS and develop standardized methods to assess it in practice.** This would allow to guide research and increase comparability

of studies performed in different countries. In addition, this is important for clinical practice.

- **Further improvement of surveillance of disseminated LB, more specifically LNB.** Set-up of standardized surveillance in European countries in which it does not yet exist will allow better comparison of LB incidence rates between countries.
- **Research into the risk factors, cause, underlying mechanism, progression over time and possible treatments for PTLDS.**
- **Improve diagnostic methods for other tick-borne diseases.** Sensitive and specific tests are needed both in research and clinical settings.
- In a more general context; **research into improved prevention, diagnosis and treatment of LB.** This includes the development of a safe and cost-effective vaccine, development of diagnostic tests able to differentiate between past and current infection, etc. These subjects are also part of the resolution on LB in the European parliament.
- **Improved research in patients who attribute persisting non-specific symptoms to LB, without having a confirmed LB diagnosis.**

Of course also research beyond the borders of Europe is of great interest, yet, attention should be payed to differences in LB between different regions.

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Summary

Lyme borreliosis (LB) is a tick-borne infectious disease, caused by spirochete bacteria of the *Borrelia burgdorferi* sensu lato (s.l.) complex. These bacteria are transmitted to humans through the bite of hard ticks, predominantly *Ixodes ricinus* in Europe. The most common clinical manifestation of LB is an erythema migrans (EM), a red expanding rash at the site of the tick bite. If this early disease stage is left untreated, or possibly without prior symptoms, the bacteria can spread to different organs, resulting in disseminated or late LB affecting the skin, the nervous system, the joints or more rarely, the heart or the eyes. Furthermore, persisting non-specific symptoms after appropriate antibiotic treatment – such as fatigue, widespread musculoskeletal pain or cognitive difficulties – have been reported by a subset of patients. If such symptoms persist for 6 months or more, and are of such severity that they affect daily activities, this is often referred to as post-treatment Lyme disease syndrome (PTLDS).

The main objective of this thesis was to contribute to the assessment of the burden of LB and other tick-borne diseases in Belgium. Since several data that allow such an assessment have not been routinely collected in Belgium, a prospective cohort study entitled the HUMTICK study was set up. The full study protocol and its update are described in **Chapter 2** and **3** of this thesis. Between June 2016 and December 2019, patients with an EM or disseminated/late LB were included through a network of GPs and several Belgian hospitals respectively. The cohorts were followed-up for 6 to 24 months depending on the moment of inclusion. Data on patient demographics, health before LB, the initial LB diagnosis, symptoms at follow-up, impact on daily activities and the incurred costs were collected. In addition, standardized questionnaires on pain, vitality, cognitive difficulties and quality of life were added. A control group of persons without prior LB was concurrently followed up during 6 to 12 months with similar questionnaires.

In order to have reliable estimates of the incidence of the different clinical manifestations of LB in Belgium, allowing cost and burden analysis, surveillance data for EM were combined with a systematic review and a meta-analysis of existing literature on the proportional distribution of the different clinical manifestations in neighboring countries (**Chapter 4**). For the period 2015–2017, the incidence was estimated at 97.6/100,000 inhabitants (95% UI 82.0–113.0) for EM and 5.9/100,000 (95% UI 4.5-7.5) for disseminated/late LB. This corresponds to respectively 11,022 (95% UI 9,267–12,768) and 663 (95% UI 510-852) annual cases in Belgium. The estimated EM incidence in 2015–2017 was comparable to the EM incidence estimated in 2008–2009. Future studies to validate these estimates would be of great value.

In **Chapter 5,** the proportion of LB patients developing PTLDS in the prospective cohort study was estimated at 5.9% (95% CI 2.7-12.9) in EM patients and 20.9% (95% CI 6.8-64.4) in disseminated/late LB patients (risk ratio= 3.5, 95% CI 1.0-12.7, p=0.053). No significant risk factors were identified, which may be explained by small sample sizes in the study. In a comparison of symptoms between LB patients and controls, the risk of presenting at least one PTLDS-related symptom was significantly higher in EM patients compared to controls, at 6 months follow-up, however, only when impact on daily activities was not taken into account. In disseminated/late LB patients, the risk on at least one PTLDS-related symptom was higher compared to controls, at both 6 and 12 months follow-up, without or with taking into account an impact on daily activities. More specifically, pain and fatigue were identified as relevant symptoms associated with LB.

The economic burden associated with the different clinical manifestations of LB was estimated in **Chapter 6**. The total annual cost was estimated at €5.59 million (95% UI 3.82-7.98), of which €3.44 million (95% UI 2.05-5.48) was related to disseminated/late LB manifestations and €2.15 million (95% UI 1.30-3.26) to EM. The number of patients with PTLDS was too small to have representative estimates for this group, yet the costs seemed to be somewhat higher.

In **Chapter 7,** the blood of 119 patients with an EM and 14 with fever after a recent tick bite was screened for the presence of several tick-borne pathogens using multiplex PCR techniques. No infections were detected; yet, their occurrence cannot be excluded due to limitations in the methodology used and the limited number of patients in the study. The study underscored the possibility of false-positive qPCR-results and the necessity for the new development or improvement of laboratory tests for the accurate diagnosis of emerging tick-borne pathogens.

In **Chapter 8,** the findings of this thesis were placed in a broader perspective and avenues for future surveillance and research were presented.

This thesis provided important insights in the incidence of the different clinical manifestations of LB, the occurrence of non-specific symptoms after treatment and the economic burden related to the disease in Belgium, hence contributing to the assessment of the burden of LB in Belgium. Such estimates are essential to inform patients, healthcare providers and policymakers on the public health impact of this disease allowing further defining control priorities. Nevertheless, there were some limitations to the study, including a small sample size for the disseminated/late LB group and the group of patients with PTLDS in the prospective cohort study. In addition, other important information, such as an assessment of the quality of life of patients with LB and PTLDS, is still missing for Belgium.

The results of our work can also guide future surveillance and research. Since changes in the incidence of LB can be expected in the future, amongst others, due to climate change, further follow-up of the EM incidence is essential. As important annual fluctuations occur, continuous surveillance is recommended. Additional surveillance of disseminated/late LB should be implemented to allow further validation of the incidence estimates for these manifestations and further follow-up of trends over the years.

As this thesis demonstrated the occurrence of PTLDS in both patients with an EM and patients with disseminated/late LB, with a higher proportion in the latter, it highlights the need for further research into the cause, risk factors, burden and possible treatments for this syndrome. In addition, the study underscored the need for the further development and validation of standardized methods that are easily applicable in practice to assess the PTLDS case definition.

For tick-borne diseases other than LB and tick-borne encephalitis, at date, it remains impossible to determine the incidence, severity and public health risk in Belgium. In order to answer such research questions, first, more knowledge on pathogenicity is needed, case definitions should be established and sensitive and specific diagnostic tests should be further developed and made routinely available.

Résumé

La borréliose de Lyme (BL) est une maladie infectieuse transmise par les tiques, causée par des bactéries spirochètes du complexe *Borrelia burgdorferi sensu lato* (s.l.). Ces bactéries sont transmises à l'homme par la morsure de tiques dures, principalement *Ixodes ricinus* en Europe. La manifestation clinique la plus courante de la BL est un érythème migrant (EM), une lésion rouge qui s'étend progressivement à partir du site de la morsure de tique. Si ce stade précoce de la maladie n'est pas traité, ou éventuellement en l'absence de symptômes préalables, la bactérie peut se disséminer dans différents organes, entraînant une BL disséminée ou tardive affectant la peau, le système nerveux, les articulations ou plus rarement, le cœur ou les yeux. En outre, des symptômes non spécifiques persistants – tels que la fatigue, des douleurs musculo-squelettiques généralisées ou des troubles cognitifs – après un traitement antibiotique adapté ont été signalés par un sous-ensemble de patients. Si ces symptômes persistent pendant 6 mois ou plus, et sont d'une sévérité telle qu'ils affectent les activités quotidiennes, on parle souvent de syndrome post-traitement de la maladie de Lyme (post-treatment Lyme disease syndrome/PTLDS).

L'objectif principal de cette thèse était de contribuer à l'évaluation du fardeau de la BL et d'autres maladies transmises par les tiques en Belgique. Comme plusieurs données permettant une telle évaluation n'ont pas été collectées de façon routinière en Belgique, une étude de cohorte prospective intitulée HUMTICK a été mise en place. Le protocole complet de l'étude et sa mise à jour sont décrits dans les **chapitres 2** et **3** de cette thèse. Entre juin 2016 et décembre 2019, les patients présentant un EM ou une BL disséminée/tardive ont été inclus respectivement via un réseau de médecins généralistes et de plusieurs hôpitaux belges. Les cohortes ont été suivies pendant 6 à 24 mois selon le moment de

l'inclusion. Des données sur les caractéristiques démographiques des patients, leur état de santé avant la BL, le diagnostic initial de la BL, les symptômes lors du suivi, l'impact sur les activités quotidiennes et les coûts engendrés ont été collectés. En outre, des questionnaires standardisés sur la douleur, la vitalité, les troubles cognitifs et la qualité de vie ont été ajoutés. Un groupe témoin de personnes indemnes de la BL a été suivi simultanément pendant 6 à 12 mois avec des questionnaires similaires.

Afin de disposer d'estimations fiables de l'incidence des différentes manifestations cliniques de la BL en Belgique, permettant une analyse des coûts de la prise en charge et du fardeau de la maladie, les données de surveillance de l'EM ont été combinées avec une revue systématique et une méta-analyse de la littérature existante sur la distribution proportionnelle des différentes manifestations cliniques dans les pays voisins (**chapitre 4**). Pour la période 2015–2017, l'incidence a été estimée à 97,6/100 000 habitants (95 % UI 82,0-113,0) pour l'EM et 5,9/100 000 (95 % UI 4,5-7,5) pour la BL disséminée/tardive. Cela correspond respectivement à 11 022 (95 % UI 9 267-12 768) et 663 (95 % UI 510-852) cas annuels en Belgique. L'incidence estimée de l'EM en 2015–2017 était comparable à l'incidence de l'EM estimée en 2008–2009. De futures études visant à valider ces estimations seraient d'une grande utilité.

Au **chapitre 5**, la proportion de patients avec la BL développant le PTLDS dans l'étude de cohorte prospective a été estimée à 5,9 % (IC 95 % 2,7-12,9) chez les patients avec un EM et 20,9 % (IC 95 % 6,8-64,4) chez les patients avec une BL disséminée/tardive (risque relatif = 3,5 ; IC 95 % 1,0-12,7 ; p=0,053). Aucun facteur de risque significatif n'a été identifié, ce qui peut s'expliquer possiblement par la petite taille des échantillons de l'étude. Dans une comparaison des symptômes entre les patients avec BL et le groupe témoin, le risque de présenter au moins un

symptôme lié au PTLDS était significativement plus élevé chez les patients EM par rapport au groupe témoin, à 6 mois de suivi, uniquement lorsque l'impact sur les activités quotidiennes n'était pas pris en compte. Chez les patients avec BL disséminée/tardive, le risque de présenter au moins un symptôme lié au PTLDS était plus élevé par rapport au groupe témoin, au suivi de 6 et 12 mois, avec ou sans la prise en compte de l'impact sur les activités quotidiennes. Plus spécifiquement, la douleur et la fatigue ont été identifiées comme des symptômes associés au BL.

Le fardeau économique associé aux différentes manifestations cliniques de la BL a été estimé au **chapitre 6**. Le coût annuel total a été estimé à 5,59 millions d'euros (95 % UI 3,82-7,98), dont 3,44 millions d'euros (95 % UI 2,05-5,48) étaient liés aux manifestations disséminées/tardives de la BL et 2,15 millions d'euros (95 % UI 1,30-3,26) à l'EM. Le nombre de patients atteints de PTLDS était trop faible pour avoir une estimation représentative pour ce groupe, mais les coûts semblaient être un peu plus élevés.

Au **chapitre 7**, le sang de 119 patients présentant un EM et de 14 autres ayant de la fièvre après une récente morsure de tique a été analysé à l'aide de tests PCR multiplex pour détecter la présence de plusieurs agents pathogènes transmis par les tiques. Aucune infection n'a été détectée ; cependant, on ne peut pas exclure leur présence en raison des limites de la méthodologie utilisée et du nombre limité de patients dans l'étude. L'étude a souligné la possibilité de résultats faussement positifs de la qPCR et la nécessité de développer ou d'améliorer les tests de laboratoire pour le diagnostic précis des agents pathogènes émergents transmis par les tiques.

Au **chapitre 8**, les résultats de cette thèse ont été placés dans une perspective plus large et des pistes de surveillance et de recherche futures ont été présentées.

Cette thèse a fourni des informations importantes sur l'incidence des différentes manifestations cliniques de la BL, la survenue de symptômes non spécifiques après le traitement et la charge économique liée à la maladie, contribuant ainsi à l'évaluation du fardeau de la BL en Belgique. De telles estimations sont essentielles pour informer les patients, les prestataires de soins de santé et les décideurs politiques sur l'impact de cette maladie sur la santé et pour mieux définir les actions prioritaires à mener pour contrôler la maladie. Néanmoins, l'étude présente certaines limites, notamment la petite taille de l'échantillon pour le groupe de patients atteints de BL disséminée/tardive et le groupe de patients atteints de PTLDS dans l'étude de cohorte prospective. En outre, d'autres informations importantes, telles que l'évaluation de la qualité de vie des patients atteints de BL et de PTLDS, manquent encore pour la Belgique.

Les résultats de cette thèse peuvent également orienter la surveillance et la recherche futures. Comme on peut s'attendre à des changements dans l'incidence de la BL à l'avenir, entre autres, en raison du changement climatique, il est essentiel de poursuivre le suivi de l'incidence de l'EM. Comme d'importantes fluctuations annuelles se produisent, une surveillance continue est recommandée. Une surveillance supplémentaire des BL disséminées/tardives devrait être mise en place pour permettre une validation supplémentaire des estimations de l'incidence de ces manifestations cliniques et un suivi supplémentaire des tendances au fil des ans.

Comme cette thèse a démontré la présence de PTLDS à la fois chez les patients atteints d'un EM et chez les patients atteints d'une BL disséminée/tardive, avec une proportion plus élevée chez ces derniers, cela souligne la nécessité de poursuivre les recherches sur la cause, les facteurs de risque, le fardeau et les traitements possibles de ce syndrome. En outre, l'étude souligne la nécessité de poursuivre le

développement et la validation de méthodes standardisées facilement applicables dans la pratique pour évaluer la définition de cas de PTLDS.

Pour les maladies transmises par les tiques autres que la BL et l'encéphalite à tiques, il est à ce jour impossible de déterminer l'incidence, la gravité et le risque pour la santé publique en Belgique. Afin de répondre à ces questions de recherche, il faut d'abord approfondir les connaissances sur la pathogénicité, des définitions de cas doivent être établies et des tests de diagnostic sensibles et spécifiques doivent être développés et rendus disponibles dans le cadre d'une procédure diagnostique de routine.

Samenvatting

Lyme borreliose (LB) is een tekenoverdraagbare infectieziekte veroorzaakt door bacteriën van het complex *Borrelia burgdorferi* sensu lato (s.l.), behorend tot de familie van spirocheten. Deze bacteriën worden op de mens overgedragen door de beet van harde teken, in Europa voornamelijk *Ixodes ricinus*. De meest voorkomende klinische manifestatie van LB is een erythema migrans (EM), een rode, zich uitbreidende huiduitslag op de plaats van de tekenbeet. Wanneer dit vroege ziektestadium onbehandeld blijft, of eventueel zonder voorafgaande symptomen, kan de bacterie zich verspreiden naar verschillende organen, wat resulteert in gedisseminеerde of late LB met aantasting van de huid, het zenuwstelsel, de gewrichten of, meer zeldzaam, het hart of de ogen. Bovendien worden er door een deel van de patiënten persisterende niet-specifieke symptomen - zoals vermoeidheid, wijdverspreide musculoskeletale pijn of cognitieve problemen - gerapporteerd na correcte antibiotica behandeling. Als dergelijke symptomen 6 maanden of langer aanhouden en zo ernstig zijn dat ze een impact hebben op dagelijkse activiteiten, wordt dit vaak als “post-treatment Lyme disease syndrome” (PTLDS) beschreven.

Het hoofddoel van deze thesis was om bij te dragen aan de evaluatie van de ziektelast van LB en andere tekenoverdraagbare ziekten in België. Aangezien verschillende gegevens, die nodig zijn voor een dergelijke evaluatie, niet routinematig verzameld werden in België, werd een nieuwe prospectieve cohortstudie, de HUMTICK-studie, opgezet. Het volledige studieprotocol en een update ervan worden beschreven in **hoofdstuk 2** en **3** van deze thesis. Tussen juni 2016 en december 2019 werden patiënten met een EM of gedisseminеerde/late LB geïncludeerd in de studie via een netwerk van respectievelijk huisartsen en verschillende Belgische ziekenhuizen. De cohorten werden 6 tot 24 maanden opgevolgd, afhankelijk van het moment van inclusie. Er werden demografische gegevens van de patiënten verzameld, gegevens over de gezondheidstoestand vóór LB, over de initiële LB-diagnose, de symptomen bij

follow-up, de impact op de dagelijkse activiteiten en de gemaakte kosten. Bovendien werden gestandaardiseerde vragenlijsten over pijn, vermoeidheid, cognitieve problemen en kwaliteit van leven toegevoegd. Een controlegroep van personen zonder LB werd gelijktijdig opgevolgd met vergelijkbare vragenlijsten gedurende 6 tot 12 maanden.

Om betrouwbare schattingen te hebben van de incidentie van de verschillende klinische manifestaties van LB in België, die een verdere kosten- en ziektelast-analyse toelaten, werden surveillancegegevens voor EM gecombineerd met een systematische review en meta-analyse van bestaande literatuur over de proportionele verhouding van andere klinische manifestaties ten opzichte van EM in de buurlanden van België (**hoofdstuk 4**). Voor de periode 2015–2017 werd de incidentie geschat op 97,6/100.000 inwoners (95% UI 82,0-113,0) voor EM en op 5,9/100.000 (95% UI 4,5-7,5) voor gedissemineerde/late LB. Dit komt overeen met respectievelijk 11.022 (95% UI 9.267-12.768) en 663 (95% UI 510-852) jaarlijkse gevallen in België. De geschatte EM-incidentie in 2015–2017 was vergelijkbaar met de geschatte EM-incidentie in 2008–2009. Toekomstige studies om deze schattingen te valideren zijn belangrijk.

In **hoofdstuk 5** werd het aandeel LB-patiënten dat PTLDS ontwikkelde in de prospectieve cohortstudie geschat op 5,9% (95% CI 2,7-12,9) in EM-patiënten en 20,9% (95% CI 6,8-64,4) in gedissemineerde/late LB-patiënten (relatief risico = 3,5; 95% CI 1,0-12,7; p=0,053). Er werden geen significante risicofactoren geïdentificeerd, wat mogelijk verklaard kan worden door de kleine steekproefgrootte in de studie. Bij een vergelijking van de symptomen tussen LB-patiënten en de controlegroep was het risico op ten minste één PTLDS-gerelateerd symptoom significant hoger bij EM-patiënten dan bij de controlegroep zonder LB, op 6 maanden follow-up, alleen wanneer geen rekening werd gehouden met de impact op dagelijkse activiteiten. Bij patiënten met gedissemineerde/late LB was het risico op ten minste één PTLDS-gerelateerd symptoom hoger in vergelijking met de controlegroep, zowel op 6 als 12 maanden follow-up, met of zonder rekening te houden met een impact op de dagelijkse

activiteiten. Meer specifiek werden pijn en vermoeidheid geïdentificeerd als relevante symptomen geassocieerd met LB.

De economische last van de verschillende klinische manifestaties van LB werd geschat in **hoofdstuk 6**. De totale jaarlijkse kosten werden geschat op €5,59 miljoen (95% UI 3,82-7,98), waarvan €3,44 miljoen (95% UI 2,05-5,48) gerelateerd was aan gedissemineerde/late LB-manifestaties en €2,15 miljoen (95% UI 1,30-3,26) aan EM. Het aantal patiënten met PTLDS was te klein om representatieve schattingen voor deze groep te kunnen maken, echter leken de kosten iets hoger te liggen.

Hoofdstuk 7 beschrijft de resultaten van een studie waarin het bloed van 119 patiënten met een EM en 14 patiënten met koorts na een recente tekenbeet gescreend werd op de aanwezigheid van verschillende tekenoverdraagbare pathogenen met behulp van multiplex PCR-technieken. Er werden geen infecties gevonden; toch kan het voorkomen ervan niet worden uitgesloten vanwege beperkingen in de gebruikte methodologie en het beperkte aantal patiënten in de studie. De studie benadrukt de kans op vals-positieve qPCR-resultaten en de noodzaak voor de verbetering en nieuwe ontwikkeling van laboratoriumtesten voor de accurate diagnose van opkomende tekenoverdraagbare pathogenen.

In **hoofdstuk 8** werden de bevindingen van deze thesis in een breder perspectief geplaatst en werden pistes voor toekomstige surveillance en onderzoek voorgesteld.

Deze thesis verschaft belangrijke inzichten in de incidentie van de verschillende klinische manifestaties van LB, het voorkomen van aspecifieke symptomen na behandeling en de economische last gerelateerd aan de ziekte in België, en draagt op die manier bij tot de evaluatie van de ziektelast van LB in België. Dergelijke schattingen zijn van essentieel belang om patiënten, zorgverstrekkers en beleidsmakers te informeren over de impact van deze ziekte op de (volks)gezondheid en om de prioriteiten voor de bestrijding van de ziekte verder te bepalen. Echter waren er ook enkele beperkingen aan de studie, waaronder een kleine steekproefgrootte voor de

gedissemineerd/late LB-groep en de groep patiënten met PTLDS in de prospectieve cohortstudie. Bovendien ontbreekt voor België nog andere belangrijke informatie, zoals een beoordeling van de levenskwaliteit van patiënten met LB en PTLDS.

De resultaten van deze thesis kunnen ook richting geven aan toekomstige surveillance en onderzoek. Aangezien veranderingen in de incidentie van LB in de toekomst verwacht kunnen worden, onder andere door klimaatsveranderingen, is verdere opvolging van de EM-incidentie essentieel. Omwille van belangrijke jaarlijkse schommelingen, wordt continue surveillance aanbevolen. Bijkomende surveillance van gedissemineerde/late LB moet worden uitgevoerd om een verdere validatie van de incidentieschattingen voor deze manifestaties en verdere opvolging van trends doorheen de jaren mogelijk te maken.

Aangezien deze thesis het voorkomen van PTLDS aantoonde bij zowel patiënten met een EM als patiënten met gedissemineerde/laattijdige LB, met een hoger aandeel bij die laatste, benadrukt het de noodzaak van verder onderzoek naar de oorzaak, risicofactoren, ziektelast en mogelijke behandelingen voor dit syndroom. Bovendien onderstreept de studie de noodzaak voor de verdere ontwikkeling en validering van gestandaardiseerde methoden, die in de praktijk gemakkelijk toepasbaar zijn, om de gevalsdefinitie van PTLDS toe te passen.

Voor andere tekenoverdraagbare ziekten dan LB en tekenencefalitis, is het tot op heden onmogelijk om de incidentie, de ernst en het risico voor de volksgezondheid in België te bepalen. Om dergelijke onderzoeksvragen te kunnen beantwoorden, is er eerst meer kennis nodig over de pathogeniteit, moeten gevalsdefinities worden opgesteld en moeten accurate diagnostische testen verder ontwikkeld en routinematig beschikbaar gesteld worden.

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Courses attended

- *Generalized Linear Models (Distance Learning)*, 1e master Biostatistics, at Hasselt University, Feb-June 2019.
- *Systematic review part II*, by CEBAM, at Leuven, 17-18 Jan. 2018.
- *Burden of disease en de Disability-Adjusted Life Year parameter*, at WIV-ISP, 5-6 Oct. 2016.
- *Economische evaluaties van medische interventies*, by ME-TA, at Leuven, 13-15 Sept. 2016.
- *Introduction to "R"*, by SMCS UCL, at Louvain-la-Neuve, 7-9 Sept. 2016.
- *Questionnaire Design and Implementation for Web Surveys*, by Social Research Association (SRA), at WIV-ISP, 27-28 Apr. 2016.
- *SAS Enterprise Guide 1: querying and reporting (EG 7.1)*, by SAS Institute, at Tervuren, 25-27 Feb. 2016.
- *Blaise training (questionnaire building)*, by Blaise CBS Nl, at WIV-ISP, 14-16 Dec. 2015.

Scientific conferences

- *Doctoral day in public health*, EDT/SPSS, 3 Dec. 2019, UCLouvain, Louvain-la-Neuve.
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- *Wildlife crossing borders*, BWDS & DSWH, 7 Oct. 2016, ITM, Antwerp.
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- *Teken en Tekenziekten in Vlaanderen*, Agentschap zorg & gezondheid, 22 Sept. 2016, Gent.

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