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

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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 2015–2017. 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.

1. 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 (Stanek et al., 2012; Steere et al., 2016). 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) (Stanek et al., 2012; Steere et al., 2016).

In order to assess the health burden and costs of LB, data on the incidence of all the different clinical manifestations are required

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(Geebelen et al., 2017; Mihajlovic et al., 2017; van den Wijngaard et al., 2015, 2017b). 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) (Vanthomme et al., 2012). 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 (Bleyenheuft et al., 2015; Rebolledo et al., 2017). 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 (Stanek et al., 2011), 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, health care systems, case definitions, and laboratory tests used (European Centre for Disease Prevention and Control (ECDC), 2012; Smith and Takkinen, 2006). Nevertheless, comparing the proportional occurrence of the different clinical manifestations between countries with a similar level of health care 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 (Coipan et al., 2016; Hofhuis et al., 2015b; Jahfari et al., 2017; Steere et al., 2016; Strnad et al., 2017). 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 (Coipan et al., 2016; Jahfari et al., 2017; Rudenko et al., 2011; Stanek and Reiter, 2011; Stanek et al., 2012). 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) (Strnad et al., 2017).

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.

2. Methods

2.1. 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 (Boffin et al., 2017). 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 (Centers for Disease Control and Prevention, 2018; Stanek et al., 2011), 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 2.5th and 97.5th percentile of the uncertainty distribution. All calculations were done in R version 3.5.0 (R Core Team, 2018).

2.2. Systematic review and meta-analysis

2.2.1. 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 (Moher et al., 2009) (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, Robert Koch-Institut, 2009).

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.

2.2.2. Quality assessment

The quality assessment of included studies focused on adaptations made to the European case definitions (Stanek et al., 2011), 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.

2.2.3. 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.

2.2.4. 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 (Riley et al., 2011). 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 (R Core Team, 2018; Viechtbauer, 2010).

2.3. 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 (Fig. 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 (R Core Team, 2018.

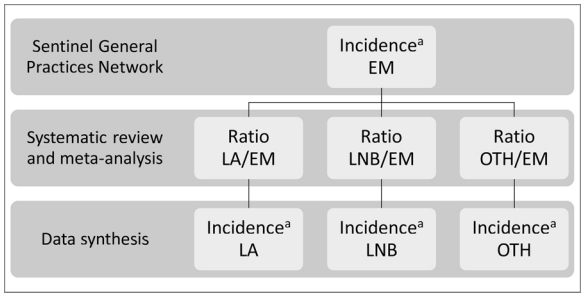

Fig. 1. Overview of methods used to quantify the incidence of clinical manifestations of Lyme borreliosis.
[a]Incidence in Belgium.
EM: erythema migrans; LA: Lyme arthritis; LNB: Lyme neuroborreliosis; OTH: other manifestations.

3. Results

3.1. 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. Fig. 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.

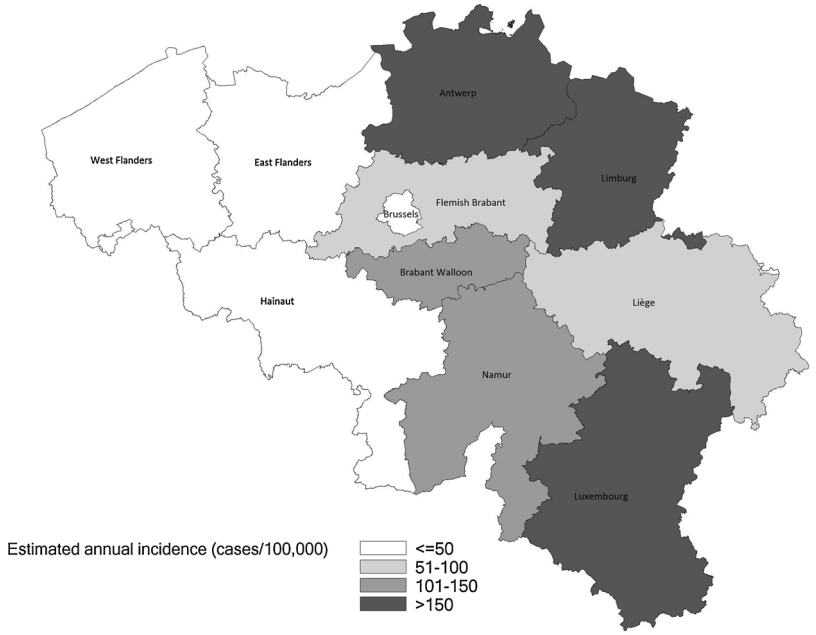

Fig. 2. Estimated annual incidence of erythema migrans (cases/100,000 inhabitants) per province, Sentinel General Practices network, Belgium, 2015–2017.

Women represented 49.6% of the cases and the incidence peaked in the 60–69 years age group, especially in men (Fig. 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.

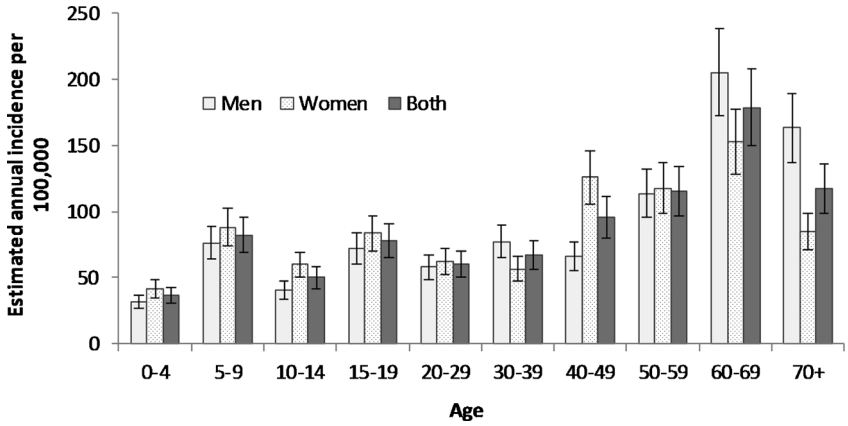

Fig. 3. Estimated annual incidence of erythema migrans (cases/100,000 inhabitants) by age and gender, Sentinel General Practices network, Belgium, 2015–2017.

3.2. Systematic review and meta-analysis

3.2.1. 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 (Hofhuis et al., 2015b; Vandenesch et al., 2014; Wilking and Stark, 2014). Studies were excluded based on: double reporting (Hofhuis et al., 2015a; van den Wijngaard et al., 2015), data (partly) older than 2008 (Blanc, 2009; Dillon et al., 2010; Fulop and Poggensee, 2008; Halsby et al., 2014), review article (Dubrey et al., 2014), not reporting on EM, LA and LNB (Bleyenheuft et al., 2015; Klier et al., 2013; Marcu et al., 2013; Vanthomme et al., 2012) and not in line with the European case definitions (Slack et al., 2011). In addition, data from two surveillance reports were included through the search of public health websites of the concerned countries (Heinzinger et al., 2017; Serre et al., 2017). Since one report described two surveillance systems, three data sets could be added. Duplicate data were excluded. A PRISMA flowchart is provided in Fig. 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.

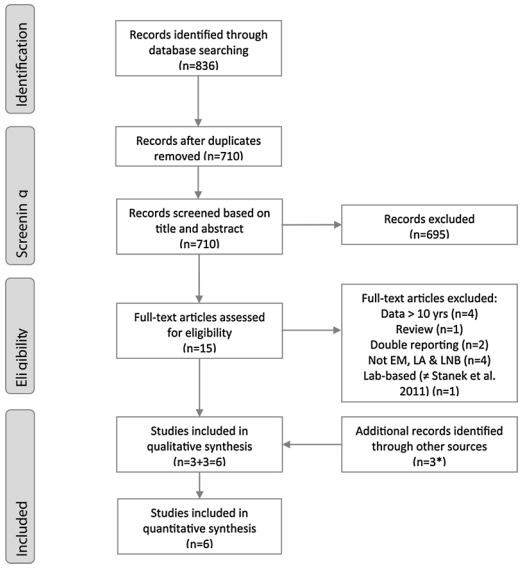

Fig. 4. Study selection flowchart.
*One record provided data from two studies, included as two studies.

3.2.2. Study characteristics

The selected data were from the Netherlands (n = 1) (Hofhuis et al., 2015b), Germany (n = 3) (Heinzinger, 2016; Wilking and Stark, 2014) and France (n = 2) (Serre et al., 2017; Vandenesch et al., 2014). 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 (Hofhuis et al., 2015b) and prospectively through weekly reporting by the *Sentinelles* GP surveillance network in France (Vandenesch et al., 2014). In one area in France, Franche-Comté (Serre et al., 2017), and one area in Germany, Bavaria (Lyme Disease Incidence-Study: LYDI-Sentinel) (Heinzinger et al., 2017), 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 (Wilking and Stark, 2014) and for physicians in Bavaria (Heinzinger et al., 2017). 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.

3.2.3. Quality assessment

As requested in the inclusion criteria, case definitions used in the studies included were in line with the European case definitions (Stanek et al., 2011), 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 (Hofhuis et al., 2015b). The French study by Vandenesch et al. (2014), the LYDI-Sentinel study in Bavaria (Heinzinger et al., 2017) and the study in Franche-Comté (Serre et al., 2017) 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 (Binder et al., 2015). 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 (Heinzinger et al., 2017; Hofhuis et al., 2015b; Serre et al., 2017; Vandenesch et al., 2014; Wilking and Stark, 2014). None of the studies were excluded because of poor quality.

3.2.4. 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., 2015b *(+ personal communication)*	11,442	234	198	156
Vandenesch et al., 2014	309	10	6	7[b]
Wilking and Stark, 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.

3.2.5. 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.

The results of a scenario analysis excluding the studies based on mandatory notification are presented in Appendix D, Table D1–D3 . No important differences were observed.

Table 2
Ratios of LNB, LA 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.

3.3. 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, LNB, LA 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 (9267–12,768)
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 (9821–13,537)

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.

4. 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). Other data sources also did not indicate a significant increase of LB in the Belgian population over the past decade (Bleyenheuft et al., 2015; De Keukeleire et al., 2017; Rebolledo et al., 2017). 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 (Bleyenheuft et al., 2015; Rebolledo et al., 2017; Sciensano, 2018). Important regional differences also exist in other European countries (Hofhuis et al., 2016; Vandenesch et al., 2014; Wilking and Stark, 2014).

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, even joined in one group, other LB manifestations are relatively rare (ratio of 0.014). 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 (Altpeter et al., 2013; Berglund et al., 1995; Cimmino, 1998; Huppertz et al., 1999; Letrilliart et al., 2005; Mehnert and Krause, 2005; Mihajlovic et al., 2017; Priem et al., 2003; Rizzoli et al., 2011). The only available data on the frequency of the different manifestations for Belgium are reported by a study by Bigaignon et al. in 1989 (Bigaignon et al., 1989). 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 (Dillon et al., 2010; Dryden et al., 2015; Lipsker et al., 2001; Lovett et al., 2008; Marcu et al., 2013). 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 (Wormser et al., 2006). 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 (Strnad et al., 2017) 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 (van den Wijngaard et al., 2017a). 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 (Hofhuis et al., 2015a, b; Vandenesch et al., 2014). In practice this might not always be the case and there might still be an overestimation of the proportion of EM (Vandenesch et al., 2014). 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 (Serre et al., 2017; Vandenesch et al., 2014); 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 and Stark, (2014)), but in practice it could be prone to underreporting (Jones et al., 2012; Naleway et al., 2002; Schiffman et al., 2018; van den Wijngaard et al., 2017a). 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 (Huppertz et al., 1999). 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 (Schiffman et al., 2018; White et al., 2018; Wilking and Stark, 2014). Another limitation from the study by Wilking and Stark, (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 (Smith and Takkinen, 2006). 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 (European Centre for Disease Prevention and Control (ECDC), 2012; Hubalek, 2009; Smith and Takkinen, 2006). 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 (Heinzinger et al., 2017; Serre et al., 2017; Vandenesch et al., 2014). 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 (Vandenesch et al., 2014). The German studies also only considered early LNB and not late LNB cases (Heinzinger et al., 2017; Wilking and Stark, 2014). As the latter is extremely rare in Europe (< 2-5% of all LNB), this was not adjusted for in the meta-analysis (Koedel et al., 2015; Mygland et al., 2010). 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,…) (Stanek et al., 2011), 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.

5. 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.

Declaration of interest

None.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at https://doi.org/10.1016/j.ttbdis.2018.12.007.

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