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ABSTRACT

Lyme neuroborreliosis (LNB) has recently been added to the list of diseases under the European Union epidemiological surveillance in order to obtain updated information on incidence. The goal of this study was to identify temporal (yearly) variation, high risk geographical regions and risk groups, and seasonal variation for LNB in Denmark.

This cohort-study investigated Danish patients (n= 2,791) diagnosed with LNB (defined as a positive *Borrelia burgdorferi* sensu lato (s.l.) intrathecal antibody test) between 1996-2015. We calculated incidence and incidence ratios of LNB by comparing 4-yr groups of calendar-years, area of residency, sex and age, income and education groups, and the number of new LNB cases per month.

The incidence of LNB was 2.2 per 100,000 individuals and year in 1996-1999, 2.7 in 2004-2007 and 1.1 per 100,000 individuals in 2012-2015. Yearly variations in LNB incidence were similar for most calendar-year groups. LNB incidence was highest in Eastern Denmark and among males and individuals who were 0-14 yrs old, who had a yearly income of >449,000 DKK, and who had a Master's degree or higher education. The number of LNB cases was highest from July to November ($p < 0.001$).

In conclusion, based on Danish nationwide data of patients with positive *B. burgdorferi* s.l. intrathecal antibody index (1996-2015) the incidence of LNB was found to increase until 2004-2007 but thereafter to decline. European surveillance studies of Lyme borreliosis should be encouraged to monitor the incidence trend.

1. INTRODUCTION

Lyme borreliosis, a tick-borne disease caused by the spirochetes of the *Borrelia burgdorferi* sensu lato (s.l.) complex is the most prevalent vector-borne infection in Europe (Stanek et al., 2012). Due to climatic and environmental changes, the incidence of tick-borne disease is expected to increase, and it has been suggested that Lyme borreliosis will become a more prominent health concern. However, as it is recognized that surveillance across European countries is heterogenous Lyme neuroborreliosis (LNB) has since 2018 been included on the list of diseases under the European Union epidemiological surveillance by the European Commission (The Lancet, 2018) in order to achieve more comprehensive information of the incidence of Lyme borreliosis at the European level.

In Denmark LNB has been a mandatory clinical notifiable disease since 1991. However, laboratory-based surveillance based on positive tests for *B. burgdorferi* s.l. intrathecal antibody index with electronic data-transfer has been shown to be more complete than surveillance based on manually processed notification and the hospital discharge databases registers (Dessau et al., 2015; Septfons et al., 2019). Furthermore, studies of surveillance of LNB based on positive test for *B. burgdorferi* s.l. intrathecal antibody index only reported a low risk of including misclassified cases (Dahl et al., 2019; Hansen and Lebech, 1992).

Potential changes in LNB incidence over time (years) in Denmark remains uninvestigated. Further identification of groups at risk for acquiring LNB will be of interest to the public and health care providers. We used an established Danish nationwide cohort of patients with LNB to investigate temporal changes in LNB incidence over time and to investigate whether LNB incidence differ according to geography, age, sex, or socioeconomic factors. Lastly, we investigated the seasonal variations of LNB incidence.

2. METHODS

2.1 Setting and data sources

In the years of study inclusion, Denmark had a population of 5.2 million to 5.7 million individuals (*Statbank Denmark*, 2019). Tax-supported health care is provided free of charge to all Danish residents (Schmidt et al., 2019). The unique 10-digit personal identification number assigned to all Danish residents at birth or upon immigration was used to track individuals in the Danish national health and administrative registries (Schmidt et al., 2019). Data on *B. burgdorferi* s.l. intrathecal antibody index were extracted from data files obtained from the Danish Departments of Microbiology laboratories that performed this test (see Supplementary Appendix). Additional data were extracted from the Danish Civil Registration system, the Building and Housing Register, the Income Statistics Register and the Danish Educational Attainment Registry (see Supplementary Appendix). We extracted data on the general Danish population numbers according to calendar-year, municipality of residence, sex, age, yearly income and highest educational attainment from Statistics Denmark (see Supplementary Appendix).

2.2 Study population

LNB patient cohort: We identified all Danish residents with a positive test for *B. burgdorferi* s.l. intrathecal antibody index during the period between 1 January 1996 and 31 December 2015, based on data files obtained from all Danish Departments of Microbiology that performed the test during the time period. *B. burgdorferi* s.l. intrathecal antibody index was measured by capture enzyme-linked immunosorbent assays (ELISA) that uses native purified flagellum from *Borrelia* (strain DK1) as antigen (Hansen and Lebech, 1991). The antibody index had been calculated using the formula: (ODcsf/ODserum)*(ODcsf − ODserum), with ODcsf and ODserum representing the optical density in cerebrospinal fluid (CSF) and sera, respectively. An antibody index ≥0.3 is regarded as positive.

Inclusion date for LNB patients was the date of lumbar puncture. The Danish cohort of LNB patients and methodology for testing has been described previously (Haahr et al., 2019; Obel et al., 2018).

2.3 Statistical analysis

<u>*Incidence – temporal changes*</u>

To investigate LNB changes over time (years) we grouped Danish resident and LNB patients according to calendar-year (1996-1999, 2000-2003, 2004-2007, 2008-2011, and 2012-2015). For each of these calendar year-periods, we divided the number of LNB cases with the number of inhabitants at risk at 1 January to estimate the average yearly incidence and incidence ratio (IR) and corresponding 95% confidence interval (CI), with the calendar year-period with the lowest incidence serving as reference. We further examined whether LNB changed over time in the demographic subgroups represented by geographical area of residence (East Zealand, North Zealand, Southwest Zealand, Funen, South Jutland, Mid Jutland, Northwest Jutland, North Jutland or Bornholm), sex (male or female), age (0-<15 years, 15-<30 years, 30-<45 years, 45-<60 years or ≥ 60 years), yearly income (<150,000 DKK, 150,000-<250,000 DKK, 250,000-<450,000 DKK or ≥ 450,000 DKK) and highest educational attainment (less than Bachelor's degree, Bachelor's degree or higher than Bachelor's degree). The geographical areas were defined according to municipalities (see Supplementary Table 1 and Supplementary Figure 1).

Incidence - demographic

Danish residents and LNB patients were grouped according to geographical area of residence, sex, age, yearly income and highest educational attainment. We identified the total number of Danish residents at risk at 1 January each year between 1996-2015 according to geographical area of residency, sex, age, yearly income and educational level. We divided the total number of LNB cases between 1996-2015 with the total number of inhabitants at risk at 1 January every year between 1996-2015 according to geographical area of residency, sex, age, yearly income and highest educational attainment to estimate average yearly incidence and IR and corresponding 95% CI with the category with the lowest incidence serving as reference.

Incidence – seasonal variation

We calculated the number of LNB patients with inclusion dates defined as the date of lumbar puncture with a positive intrathecal antibody index test for each calendar-month to estimate the number of new LNB cases per calendar-month. We ascertained difference in LNB incidence between calendar-months and performed a chi-square test to investigate for statistical differences with a significance level of $p < 0.05$. We used SPSS Statistics, version 25 (SPSS, Inc., Chicago, Illinois, USA) and R version 3.5.1 for all analysis.

2.4 Regulatory compliance

The study was approved by the Danish Data Protection Agency and the National Board of Health (RH-2015-285, I-Suite no.: 04297). An approval from the local Ethics Committee is not needed for this type of study in Denmark.

3. RESULTS

We identified a total of 2,791 LNB patients with a first-time positive test for *B. burgdorferi* s.l intrathecal antibody index between 1 January 1996 and 31 December 2015. The average incidence for the entire study period was 2.6 per 100,000 individuals per year. The median age of LNB patients was 45.8 years and the proportion of males was 56.8 % (Table 1).

3.1 *Incidence – temporal changes*

The incidence of LNB increased nationwide from the calendar year-period 1996-1999 (2.2 LNB cases per 100,000 individuals per year) to 2004-2007 (3.3 LNB cases per 100,000 individuals per year), but thereafter declined to 1.8 LNB cases per 100,000 individuals per year during 2012-2015 (Table 1 and Figure 1). The incidence of LNB increased until 2004-2007 but thereafter declined with time for most geographical areas of residency and irrespective of sex, age, yearly income or educational level (Figure 2, Supplementary Figure 2, Supplementary Figure 3, Supplementary Figure 4 and Supplementary Figure 5).

3.2 *Incidence – demographic*

As shown in Table 1, higher average incidence of LNB was observed in North Zealand, Southwest Zealand, Funen and Bornholm compared with the area with the lowest incidence of LNB (South Jutland). The average incidence of LNB was higher in males (3.0 LNB cases per 100,000 individuals per year) compared with females (2.2 LNB cases per 100,000 individuals per year) corresponding to an IR of 1.3, 95% CI: 1.2 to 1.4 (Table 1). Moreover, the average incidence of LNB was higher in individuals aged 0-<15 years (4.2 LNB cases per 100,000 individuals per year, IR 5.4, 95% CI: 4.5 to 6.3), 45-<60 years (2.9 LNB cases 100,000 individuals per year, IR 3.7, 95% CI: 3.1 to 4.3) and 60 years or older (3.3 LNB cases per 100.00 individuals per year, IR 4.2, 95% CI: 3.5 to 5.0) compared to people aged 15-<30 years (0.8 LNB cases per 100,000 individuals per year) (Table 1).

With regards to annual income, the average incidence of LNB was highest in individuals with a yearly income of 450,000 DKK or more (3.3 LNB cases 100,000 individuals per year) compared with individuals with a yearly income between 150,000-<250,000 DKK (2.1 LNB cases per 100,000 individuals per year), corresponding to an IR of 1.6, 95% CI: 1.3 to 1.8 (Table 1). Finally, the average incidence of LNB was higher in individuals with a higher educational attainment than a bachelor’s degree (3.3 cases per 100,000 individuals per year) compared with individuals with less than a bachelor's degree (2.1 LNB cases per 100,000 individuals per year), corresponding to an IR of 1.6, 95% CI: 1.3 to 1.9 (Table 1).

3.3 *Incidence - seasonal variation*

We observed a monthly variation with the lowest number of new cases of LNB in March (2.8 cases of LNB/month) and the highest number of new cases in August (26.6 cases of LNB/month) ($p < 0.0001$) (Figure 3).

4. DISCUSSION

Our study on LNB using nationwide data of positive *B. burgdorferi* s.l. intrathecal antibody index (1996-2015) provides an updated overview of the epidemiology of LNB in Denmark and documents that the incidence of LNB has increased until 2004-2007 but thereafter declined.

The overall incidence of LNB in Denmark between 1996-2015 was 2.6 per 100,000 individuals per year. This was of the same magnitude as has been estimated in earlier studies from Denmark as well as in Belgium but higher than in Germany and France (Enkelmann et al., 2018; Geebelen et al., 2019; Septfons et al., 2019). The incidence was however half the IR described from the neighboring country Sweden that reported an overall incidence of 6.3 per 100,000 for 2014. As the Swedish incidence data also was based on positive *B. burgdorferi* s.l. intrathecal antibody index cases with the national microbiology database our data seems comparable to their data. (Dahl et al., 2019; Knudtzen et al., 2017). The variation in incidences could be due to differences in prevalence of *B. burgdorferi* s.l. in the tick *Ixodes ricinus*, climate and biomes as well as number of people residing or working in areas endemic for Lyme borreliosis (Strnad et al., 2017). However, comparisons between countries must in general be interpreted with caution due to heterogeneity among surveillance systems which impact the estimates.

The incidence of LNB is likely to differ across European countries, possibly depending on differences in geographical factors, presence and abundance of ticks, distribution of the neurotropic genospecies *Borrelia garinii* as well as differences in human behavior influencing risk of tick exposure. Comparison of incidences of LNB between European countries is however difficult as surveillance of LNB is based on different methods of data collection: physician reporting, hospital diagnoses or laboratory surveillance. We used laboratory data of positive *B. burgdorferi* s.l. intrathecal antibody index as measure for LNB cases, as this previously has been shown to be more accurate as physician reporting or hospital discharge diagnosis (Dessau et al., 2015; Septfons et al., 2019).

Using this measure, we observed an increasing incidence of LNB in Denmark from 1996-1999 to 2004-2007, but thereafter the incidence declined until the last study period 2012-2015 except for in two geographical areas. In agreement, no increase in LNB incidence was observed in France between 2005-2016 (Septfons et al., 2019) or Sweden between 2002-2014, but there were increases in a specific Swedish region between 2000-2005 (Henningsson et al., 2010; Södermark et al., 2017). Furthermore, the incidence of Lyme borrelioses was reported to increase in eastern Germany between 2002-2006 but decreased overall between 2009-2012 and did not increase between 2013-2017 (Enkelmann et al., 2018; Fülöp and Poggensee, 2008; Wilking and Stark, 2014). Changes in Lyme borreliosis incidence may also be influenced by improved awareness which could lead to a decrease in the number of patients that develop LNB and other disseminated manifestations of Lyme borreliosis. The Danish physicians may be increasingly aware of early symptoms of Lyme borreliosis and therefore promptly initiate antibiotic therapy. Also, the Danish population may have become increasingly informed of the importance of daily checks for ticks, prompt removal of ticks after exposure to avoid infection and recognizing erythema migrans especially in highly endemic areas (Jepsen et al., 2019). Therefore, our results due not necessarily reflect the overall national trend of Lyme borreliosis manifestations during the study period.

Identification of possible high incidence areas for acquiring LNB will be of interest to the public and health care professionals. We observed a significant variation in LNB incidence according to geographical area. The geographical distribution of LNB was in agreement with an estimated distribution of *I. ricinus* in Denmark as well as an estimate of incidence of *Borrelia* seropositivity among roe deer (Skarphédinsson et al., 2005).

In agreement with other studies we found an increased incidence of LNB in males (Dahl et al., 2019; Enkelmann et al., 2018; Hansen and Lebech, 1992; Södermark et al., 2017). However, erythema migrans was reported to be more common in females than males (Enkelmann et al., 2018). Females have been observed to use protective practices against ticks more often than males (Jepsen et al., 2019). Males could also be less likely to notice early signs of Lyme disease compared with females. This would lead males to develop late-stage manifestations of *B. burgdorferi* s.l. infection such as LNB more often than females.

We observed a U-shaped incidence distribution with incidence being highest in children younger than 15 yr and individuals 60 yr or older as also described by others (Dahl et al., 2019; Dessau et al., 2015; Septfons et al., 2019; Wilking and Stark, 2014). The observation may partly be explained by more intense radicular pains in middle-age and elderly patients compared with younger adults leading to hospital admission (Hansen and Lebech, 1992). It has also been suggested that young adult individuals are more likely to have a subclinical infection with the neurotropic genospecies *B. garinii* (Carlsson et al., 2018) with less prominent radiculitic pain and rarely signs of meningism. Therefore, it is possible that the actual incidence of individuals infected with *B. burgdorferi* s.l. especially in this age-group is under-recognized. Explanations for the differences between age groups could also be related to differences in outdoor activity and leisure time activities between age groups. Our estimated LNB incidence for children was slightly lower than incidence of LNB in children in the two Scandinavian countries Norway and Sweden (Henningsson et al., 2010; Øymar and Tveitnes, 2009) but comparable to another study from Sweden including 548 children with LNB from Gothenburg and surrounding municipalities (Södermark et al., 2017).

We observed a proportional increase in LNB incidence with increasing income with the highest yearly earners having the highest incidence of LNB. This is in agreement with American studies on other socioeconomic factors such as race and education (Moon et al., 2019; Springer and Johnson, 2018). An increased LNB incidence for individuals with higher attained education was observed in agreement with studies from North America (Springer and Johnson, 2018). Income and educational level would be expected to be correlated, and the high incidence of LNB in groups with either high income or high education level, could likely be explained by this correlation. Since infection is correlated with tick exposure, this variability in incidence rates among age groups and education achievements could very likely be due to differences in outdoor activities.

A seasonal variation in the monthly incidence of LNB with the incidence being highest between July and November was found and thus a close association of the seasonal activity of *I. ricinus* and the onset of LNB. This agrees with other studies on the seasonal variation of LNB in Denmark (Dessau et al., 2015; Hansen and Lebech, 1992) and other European countries (Enkelmann et al., 2018; Septfons et al., 2019) as well as studies on seasonal and climatic variation in *I. ricinus* activity (Brugger et al., 2018; Lin et al., 2019; Lindgren et al., 2000).

The major strengths of the study are the large sample size and our ability to include all Danish citizens with a proven positive *B. burgdorferi* s.l. intrathecal antibody index test over a 20-yr-period. The registry-based design was hampered by lacking access to data on cerebrospinal fluid (CSF) leucocytes counts, as the presence of CSF pleocytosis would have substantiated the diagnosis of LNB further. Thus, we may have overestimated the LNB incidence. However, as a positive *B. burgdorferi* s.l. intrathecal antibody index has a high diagnostic sensitivity for LNB we assume a low rate of misclassification of LNB cases and thus this effect is likely to be very limited (Dessau et al., 2015; Hansen, 1994; Hansen and Lebech, 1992, 1991; Henningsson et al., 2014). Factors that may have led to underestimation of LNB incidence are the lack of inclusion of (i) patients with an early LNB that have not yet have developed specific *B. burgdorferi* s.l. intrathecal antibodies and (ii) patients diagnosed and treated on clinical presentation alone without CSF investigation. The observed changes in LNB incidence over the study period could be due to changes in clinical testing practices as only positive *B. burgdorferi* s.l. intrathecal antibody index tests were used to estimate incidence of LNB. However, because the national clinical guidelines for testing and diagnosing LNB in Denmark recommend testing all suspected cases of LNB with a *B. burgdorferi* s.l. antibody index test and these guidelines have not changed during the time period covered by this study, it is unlikely that the changes in LNB incidence are due to changes in how LNB is diagnosed in Denmark (Dessau et al., 2014). Our analyses of geographical variation may be limited by the fact, that we only have access to information on place of residence, which is not necessarily the place of exposure to tick bites.

5. CONCLUSION

Based on a Danish nationwide cohort of patients with LNB defined by positive *B. burgdorferi* s.l. intrathecal antibody index and data from the Danish National registries, LNB incidence in Denmark increased from the time period 1996-1999 to 2004-2007 but thereafter declined until the last study time period in 2011-2015. The incidence of LNB was highest for individuals with residency in Eastern Denmark, males, children and individuals with high income and high educational attainment.

Conflict of interest

K. Hansen has received royalties from Thermo Fisher; R. Dessau participated in advisory board meeting Roche Diagnostics 2018 outside this work; all other authors declare no conflicts of interests and no support from any organization for the submitted work.

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TABLE 1: Incidence and incidence ratio of Lyme neuroborreliosis (LNB) stratified on 4-yr time periods, geography, sex, age, yearly income and highest attained education in Denmark, 1996-2015.

	Number of LNB cases	**Incidence of LNB per 100,000 individuals per year**	**Incidence rate ratio (95% confidence interval)**
Calendar year-period			
1996-1999	480	2.2	1.3 (1.1 to 1.4)
2000-2003	579	2.7	1.5 (1.3 to 1.7)
2004-2007	714	3.3	1.8 (1.6 to 2.1)
2008-2011	616	2.8	1.6 (1.4 to 1.8)
2012-2015	402	1.8	1 (Ref.)
Geographical area			
East Zealand	683	2.3	3.9 (2.7 to 5.7)
North Zealand	266	3.9	6.5 (4.4 to 9.7)
Southwest Zealand	420	3.6	6.2 (4.2 to 9.1)
Funen	409	4.2	7.1 (4.9 to 10.5)
South Jutland	28	0.6	1 (Ref.)
Mid Jutland	463	2.8	4.7 (3.2 to 6.9)
Northwest Jutland	26	0.6	1.1 (0.6 to 1.7)
North Jutland	425	1.8	3.0 (2.1 to 4.5)
Bornholm	71	8.2	14.0 (9.0 to 21.7)
Sex			
Men	1,584	3.0	1.3 (1.2 to 1.4)
Women	1,207	2.2	1.0 (Ref.)

TABLE 1: Continued

Age (years)			
0-<15	826	4.2	5.4 (4.5 to 6.3)
15-<30	157	0.8	1.0 (Ref.)
30-<45	400	1.7	2.2 (1.8 to 2.6)
45-<60	636	2.9	3.7 (3.1 to 4.3)
>=60	772	3.3	4.2 (3.5 to 5.0)
Yearly income*			
< 150,000 DKK	474	2.2	1.0 (0.9 to 1.2)
150,000-<250,000 DKK	526	2.1	1.0 (Ref.)
250,000-<450,000 DKK	662	2.4	1.1 (1.0 to 1.3)
≥450,000 DKK	257	3.3	1.6 (1.3 to 1.8)
Highest attained education**			
Less than bachelor's degree	1,179	2.1	1.0 (Ref.)
Bachelor's degree	322	3.2	1.5 (1.3 to 1.7)
Higher than bachelor's degree	148	3.3	1.6 (1.3 to 1.9)

*Only individuals older than 20 years

**Only individuals older than 20 years and younger than 70 years

FIGURE 1: Nationwide yearly Lyme neuroborreliosis (LNB) incidence per 100,000 individuals in Denmark

FIGURE 2: Yearly Lyme neuroborreliosis (LNB) incidence per 100,000 individuals by geographical area

FIGURE 3: Average number of new cases of Lyme neuroborreliosis (LNB) nationwide per month