

Changes in Lyme neuroborreliosis incidence in Denmark, 1996 to 2015

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1 **Title:** Changes in Lyme neuroborreliosis incidence in Denmark, 1996 to 2015

2

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55 local Ethics Committee is not needed for this type of study in Denmark.

56

57 **ABSTRACT**

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

62 This cohort-study investigated Danish patients (n= 2,791) diagnosed with LNB
63 (defined as a positive *Borrelia burgdorferi* sensu lato (s.l.) intrathecal antibody test) between 1996-
64 2015. We calculated incidence and incidence ratios of LNB by comparing 4-yr groups of calendar-
65 years, area of residency, sex and age, income and education groups, and the number of new LNB
66 cases per month.

67 The incidence of LNB was 2.2 per 100,000 individuals and year in 1996-1999, 2.7 in
68 2004-2007 and 1.1 per 100,000 individuals in 2012-2015. Yearly variations in LNB incidence were
69 similar for most calendar-year groups. LNB incidence was highest in Eastern Denmark and among
70 males and individuals who were 0-14 yrs old, who had a yearly income of >449,000 DKK, and who
71 had a Master's degree or higher education. The number of LNB cases was highest from July to
72 November ($p < 0.001$).

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

77

78 **1. INTRODUCTION**

79 Lyme borreliosis, a tick-borne disease caused by the spirochetes of the *Borrelia*
80 *burgdorferi* sensu lato (s.l.) complex is the most prevalent vector-borne infection in Europe (Stanek
81 et al., 2012). Due to climatic and environmental changes, the incidence of tick-borne disease is
82 expected to increase, and it has been suggested that Lyme borreliosis will become a more prominent
83 health concern. However, as it is recognized that surveillance across European countries is
84 heterogenous Lyme neuroborreliosis (LNB) has since 2018 been included on the list of diseases
85 under the European Union epidemiological surveillance by the European Commission (The Lancet,
86 2018) in order to achieve more comprehensive information of the incidence of Lyme borreliosis at
87 the European level.

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

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

101 2. METHODS

102 2.1 Setting and data sources

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

115

116 2.2 Study population

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

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

129

130 2.3 Statistical analysis

131 *Incidence – temporal changes*

132 To investigate LNB changes over time (years) we grouped Danish resident and LNB
133 patients according to calendar-year (1996-1999, 2000-2003, 2004-2007, 2008-2011, and 2012-
134 2015). For each of these calendar year-periods, we divided the number of LNB cases with the
135 number of inhabitants at risk at 1 January to estimate the average yearly incidence and incidence
136 ratio (IR) and corresponding 95% confidence interval (CI), with the calendar year-period with the
137 lowest incidence serving as reference. We further examined whether LNB changed over time in the
138 demographic subgroups represented by geographical area of residence (East Zealand, North
139 Zealand, Southwest Zealand, Funen, South Jutland, Mid Jutland, Northwest Jutland, North Jutland
140 or Bornholm), sex (male or female), age (0-<15 years, 15-<30 years, 30-<45 years, 45-<60 years or
141 ≥ 60 years), yearly income (<150,000 DKK, 150,000-<250,000 DKK, 250,000-<450,000 DKK or \geq
142 450,000 DKK) and highest educational attainment (less than Bachelor's degree, Bachelor's degree
143 or higher than Bachelor's degree). The geographical areas were defined according to municipalities
144 (see Supplementary Table 1 and Supplementary Figure 1).

145

146 Incidence - demographic

147 Danish residents and LNB patients were grouped according to geographical area of
148 residence, sex, age, yearly income and highest educational attainment. We identified the total
149 number of Danish residents at risk at 1 January each year between 1996-2015 according to
150 geographical area of residency, sex, age, yearly income and educational level. We divided the total
151 number of LNB cases between 1996-2015 with the total number of inhabitants at risk at 1 January
152 every year between 1996-2015 according to geographical area of residency, sex, age, yearly income
153 and highest educational attainment to estimate average yearly incidence and IR and corresponding
154 95% CI with the category with the lowest incidence serving as reference.

155

156 Incidence – seasonal variation

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

163

164 **2.4 Regulatory compliance**

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

168

169 **3. RESULTS**

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

174

175 3.1 Incidence – temporal changes

176 The incidence of LNB increased nationwide from the calendar year-period 1996-1999
177 (2.2 LNB cases per 100,000 individuals per year) to 2004-2007 (3.3 LNB cases per 100,000
178 individuals per year), but thereafter declined to 1.8 LNB cases per 100,000 individuals per year
179 during 2012-2015 (Table 1 and Figure 1). The incidence of LNB increased until 2004-2007 but
180 thereafter declined with time for most geographical areas of residency and irrespective of sex, age,
181 yearly income or educational level (Figure 2, Supplementary Figure 2, Supplementary Figure 3,
182 Supplementary Figure 4 and Supplementary Figure 5).

183

184 3.2 Incidence – demographic

185 As shown in Table 1, higher average incidence of LNB was observed in North
186 Zealand, Southwest Zealand, Funen and Bornholm compared with the area with the lowest
187 incidence of LNB (South Jutland). The average incidence of LNB was higher in males (3.0 LNB
188 cases per 100,000 individuals per year) compared with females (2.2 LNB cases per 100,000
189 individuals per year) corresponding to an IR of 1.3, 95% CI: 1.2 to 1.4 (Table 1). Moreover, the
190 average incidence of LNB was higher in individuals aged 0-<15 years (4.2 LNB cases per 100,000
191 individuals per year, IR 5.4, 95% CI: 4.5 to 6.3), 45-<60 years (2.9 LNB cases 100,000 individuals
192 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
193 per year, IR 4.2, 95% CI: 3.5 to 5.0) compared to people aged 15-<30 years (0.8 LNB cases per
194 100,000 individuals per year) (Table 1).

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

201 individuals with less than a bachelor's degree (2.1 LNB cases per 100,000 individuals per year),
202 corresponding to an IR of 1.6, 95% CI: 1.3 to 1.9 (Table 1).

203

204 3.3 Incidence - seasonal variation

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

208

209

210 **4. DISCUSSION**

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

214
215 The overall incidence of LNB in Denmark between 1996-2015 was 2.6 per 100,000
216 individuals per year. This was of the same magnitude as has been estimated in earlier studies from
217 Denmark as well as in Belgium but higher than in Germany and France (Enkelmann et al., 2018;
218 Geebelen et al., 2019; Septfons et al., 2019). The incidence was however half the IR described from
219 the neighboring country Sweden that reported an overall incidence of 6.3 per 100,000 for 2014. As
220 the Swedish incidence data also was based on positive *B. burgdorferi* s.l. intrathecal antibody index
221 cases with the national microbiology database our data seems comparable to their data. (Dahl et al.,
222 2019; Knudtzen et al., 2017). The variation in incidences could be due to differences in prevalence
223 of *B. burgdorferi* s.l. in the tick *Ixodes ricinus*, climate and biomes as well as number of people
224 residing or working in areas endemic for Lyme borreliosis (Strnad et al., 2017). However,
225 comparisons between countries must in general be interpreted with caution due to heterogeneity
226 among surveillance systems which impact the estimates.

227 The incidence of LNB is likely to differ across European countries, possibly
228 depending on differences in geographical factors, presence and abundance of ticks, distribution of
229 the neurotropic genospecies *Borrelia garinii* as well as differences in human behavior influencing
230 risk of tick exposure. Comparison of incidences of LNB between European countries is however
231 difficult as surveillance of LNB is based on different methods of data collection: physician
232 reporting, hospital diagnoses or laboratory surveillance. We used laboratory data of positive *B.*
233 *burgdorferi* s.l. intrathecal antibody index as measure for LNB cases, as this previously has been
234 shown to be more accurate as physician reporting or hospital discharge diagnosis (Dessau et al.,
235 2015; Septfons et al., 2019).

236 Using this measure, we observed an increasing incidence of LNB in Denmark from 1996-1999 to
237 2004-2007, but thereafter the incidence declined until the last study period 2012-2015 except for in
238 two geographical areas. In agreement, no increase in LNB incidence was observed in France
239 between 2005-2016 (Septfons et al., 2019) or Sweden between 2002-2014, but there were increases
240 in a specific Swedish region between 2000-2005 (Henningson et al., 2010; Södermark et al., 2017).
241 Furthermore, the incidence of Lyme borreliosis was reported to increase in eastern Germany

242 between 2002-2006 but decreased overall between 2009-2012 and did not increase between 2013-
243 2017 (Enkelmann et al., 2018; Fülöp and Poggensee, 2008; Wilking and Stark, 2014). Changes in
244 Lyme borreliosis incidence may also be influenced by improved awareness which could lead to a
245 decrease in the number of patients that develop LNB and other disseminated manifestations of
246 Lyme borreliosis. The Danish physicians may be increasingly aware of early symptoms of Lyme
247 borreliosis and therefore promptly initiate antibiotic therapy. Also, the Danish population may have
248 become increasingly informed of the importance of daily checks for ticks, prompt removal of ticks
249 after exposure to avoid infection and recognizing erythema migrans especially in highly endemic
250 areas (Jepsen et al., 2019). Therefore, our results due not necessarily reflect the overall national
251 trend of Lyme borreliosis manifestations during the study period.

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

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

264 We observed a U-shaped incidence distribution with incidence being highest in
265 children younger than 15 yr and individuals 60 yr or older as also described by others (Dahl et al.,
266 2019; Dessau et al., 2015; Septfons et al., 2019; Wilking and Stark, 2014). The observation may
267 partly be explained by more intense radicular pains in middle-age and elderly patients compared
268 with younger adults leading to hospital admission (Hansen and Lebech, 1992). It has also been
269 suggested that young adult individuals are more likely to have a subclinical infection with the
270 neurotropic genospecies *B. garinii* (Carlsson et al., 2018) with less prominent radiculitic pain and
271 rarely signs of meningism. Therefore, it is possible that the actual incidence of individuals infected
272 with *B. burgdorferi* s.l. especially in this age-group is under-recognized. Explanations for the
273 differences between age groups could also be related to differences in outdoor activity and leisure

274 time activities between age groups. Our estimated LNB incidence for children was slightly lower
275 than incidence of LNB in children in the two Scandinavian countries Norway and Sweden
276 (Henningsson et al., 2010; Øymar and Tveitnes, 2009) but comparable to another study from
277 Sweden including 548 children with LNB from Gothenburg and surrounding municipalities
278 (Södermark et al., 2017).

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

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

294 The major strengths of the study are the large sample size and our ability to include all
295 Danish citizens with a proven positive *B. burgdorferi* s.l. intrathecal antibody index test over a 20-
296 yr-period. The registry-based design was hampered by lacking access to data on cerebrospinal fluid
297 (CSF) leucocytes counts, as the presence of CSF pleocytosis would have substantiated the diagnosis
298 of LNB further. Thus, we may have overestimated the LNB incidence. However, as a positive *B.*
299 *burgdorferi* s.l. intrathecal antibody index has a high diagnostic sensitivity for LNB we assume a
300 low rate of misclassification of LNB cases and thus this effect is likely to be very limited (Dessau et
301 al., 2015; Hansen, 1994; Hansen and Lebech, 1992, 1991; Henningsson et al., 2014). Factors that
302 may have led to underestimation of LNB incidence are the lack of inclusion of (i) patients with an
303 early LNB that have not yet have developed specific *B. burgdorferi* s.l. intrathecal antibodies and
304 (ii) patients diagnosed and treated on clinical presentation alone without CSF investigation. The
305 observed changes in LNB incidence over the study period could be due to changes in clinical testing

306 practices as only positive *B. burgdorferi* s.l. intrathecal antibody index tests were used to estimate
307 incidence of LNB. However, because the national clinical guidelines for testing and diagnosing
308 LNB in Denmark recommend testing all suspected cases of LNB with a *B. burgdorferi* s.l. antibody
309 index test and these guidelines have not changed during the time period covered by this study, it is
310 unlikely that the changes in LNB incidence are due to changes in how LNB is diagnosed in
311 Denmark (Dessau et al., 2014). Our analyses of geographical variation may be limited by the fact,
312 that we only have access to information on place of residence, which is not necessarily the place of
313 exposure to tick bites.

314

315 **5. CONCLUSION**

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

322

323 **Conflict of interest**

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

327

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448 **TABLE 1:** Incidence and incidence ratio of Lyme neuroborreliosis (LNB) stratified on 4-yr time
 449 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			
<i>1996-1999</i>	480	2.2	1.3 (1.1 to 1.4)
<i>2000-2003</i>	579	2.7	1.5 (1.3 to 1.7)
<i>2004-2007</i>	714	3.3	1.8 (1.6 to 2.1)
<i>2008-2011</i>	616	2.8	1.6 (1.4 to 1.8)
<i>2012-2015</i>	402	1.8	1 (Ref.)
Geographical area			
<i>East Zealand</i>	683	2.3	3.9 (2.7 to 5.7)
<i>North Zealand</i>	266	3.9	6.5 (4.4 to 9.7)
<i>Southwest Zealand</i>	420	3.6	6.2 (4.2 to 9.1)
<i>Funen</i>	409	4.2	7.1 (4.9 to 10.5)
<i>South Jutland</i>	28	0.6	1 (Ref.)
<i>Mid Jutland</i>	463	2.8	4.7 (3.2 to 6.9)
<i>Northwest Jutland</i>	26	0.6	1.1 (0.6 to 1.7)
<i>North Jutland</i>	425	1.8	3.0 (2.1 to 4.5)
<i>Bornholm</i>	71	8.2	14.0 (9.0 to 21.7)
Sex			
<i>Men</i>	1,584	3.0	1.3 (1.2 to 1.4)
<i>Women</i>	1,207	2.2	1.0 (Ref.)

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452 **TABLE 1:** Continued

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

453 *Only individuals older than 20 years

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

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

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

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

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