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Original article

Seroprevalence of Lyme borreliosis in Finland 50 years ago*

J. Cuellar [1, 2, *], T. Dub [3, 4], J. Sane [3], J. Hytönen [1, 5]

[1] Institute of Biomedicine, Faculty of Medicine, University of Turku, Turku, Finland
[2] Turku Doctoral Programme of Molecular Medicine, Faculty of Medicine, University of Turku, Turku, Finland
[3] Department of Health Security, National Institute for Health and Welfare, Helsinki, Finland
[4] European Programme for Intervention Epidemiology Training (EPIET), European Centre for Disease Prevention and Control (ECDC), Stockholm, Sweden
[5] Laboratory Division, Clinical Microbiology, Turku University Hospital, Turku, Finland

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ABSTRACT

Objectives: Lyme borreliosis (LB) is a tick-borne infection common in Europe. In Finland, the LB seroprevalence in the healthy population was 3.9% in 2011. While the present-day seroprevalence of LB is well characterized in several European areas, there are no studies on the seroprevalence of LB before the description of the infection in the late 1970s.

Methods: We used a subset of historical serum samples ($n = 994$) collected during the Finnish Mobile Clinic Health Survey, a nationwide cross-sectional health survey of the 1960s and 1970s. All samples were screened with *Borrelia burgdorferi* whole-cell sonicate IgG ELISA. The seropositivity of the samples was further confirmed by the C6 peptide ELISA and recomBead IgG 2.0 bead immunoassay. The association of LB seropositivity with risk factors and with self-reported diseases and symptoms relating to disseminated LB were analysed by logistic regression.

Results: *B. burgdorferi* IgGs were detected in 199 of 994 analysed samples; hence, the overall seroprevalence was 20.0% (95% confidence interval: 17.6–22.6). The highest seroprevalence was observed in persons aged ≥50 years (165/696), in those currently not working (92/383), and in the regions of South and Central Finland (91/226 and 27/88, respectively). Further, perception of feeling unhealthy (129/197 versus 412/794) was higher among LB-seropositive individuals compared to LB-seronegative participants.

Conclusion: LB seroprevalence was considerably higher in Finland in the late 1960s and early 1970s than in 2011. This result questions the perception of an unprecedentedly high LB seroprevalence in present-day Europe. **J. Cuellar, Clin Microbiol Infect 2019;▪:1**

Introduction

Lyme borreliosis (LB) is a tick-borne infectious disease common in Europe and in the USA [1]. The causal bacteria belong to the *Borrelia burgdorferi* sensu lato complex (later *Borrelia*), where *B. burgdorferi* sensu stricto (ss), *B. afzelii*, and *B. garinii* are the most prevalent human pathogens [2]. The clinical manifestations of LB vary from a local skin infection (erythema migrans, EM) to various forms of the disseminated LB such as neuroborreliosis, arthritis, and chronic skin inflammation (acrodermatitis chronica atrophicans, ACA) [1].

Serology is the standard laboratory method used to support the diagnosis of patients with disseminated LB [3,4]. Immunoglobulin (Ig) M and IgG antibodies towards *Borrelia* are detected in serum of patients within 6–8 weeks after the infection [4]. In the disseminated form of LB, over 99% patients have detectable antibodies [5]. The *Borrelia* IgG antibodies can persist for 10–20 years after the active infection [6]. Hence, serology is also applied to epidemiological studies to evaluate the seroprevalence as a measure of exposure to LB infection in a defined population.

In a recent epidemiological review, the number of European LB cases was estimated to be over 200 000 patients annually [7]. Similarly, in the USA over 300 000 LB cases are reported annually

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* Corresponding author. J. Cuellar, Institute of Biomedicine, University of Turku, Kiinamyllynkatu 10, FI-20520, Turku, Finland.
E-mail address: Julia.cuellar@utu.fi (J. Cuellar).

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[8]. In Western Europe, the population-weighted incidence rate of LB has been calculated as 22 cases per 100 000 inhabitants [7]. However, the LB incidence rate, and thus also the seroprevalence, varies largely between European countries and even among different regions within one country [7]. For example, in Germany the estimated seroprevalence is 9.4%, in Belgium 1.1%, in Norway 4.0%, and in Poland 12.5% [9–12]. Furthermore, in selected groups of people with outdoor activities, such as forestry workers, farmers, and orienteers [13–17], and in residents of highly endemic geographical areas [18,19], LB seroprevalence can be as high as 20%. In our recent study using the same serological algorithm, the LB seroprevalence among the general population in Finland in 2011 was estimated as 3.9% [20].

The documented history of LB starts at the end of the 19th century, when the German physician Buchwald described a chronic skin disorder later recognized as ACA [21]. From the beginning of the 20th century, there were case reports of patients with expanding skin lesions (EM) by the Swedish physician Afzelius [22] and with nervous system involvement (neuroborreliosis) by the French neurologists Garin and Bujadoux [23]. However, only since the 1990s, epidemiological studies on LB have been conducted in Europe [24–26]. Importantly, no studies have been published on the LB seroprevalence using human serum samples collected prior to the time when *Borrelia* was identified as the LB causative bacterium in the early 1980s [27].

In this study, we report the results of LB seroprevalence among the general population in Finland during the years 1968–1972, and of the related risk factors using serum samples and background data collected during a cross-sectional nationwide study. The results of this study are discussed in relation to the seroprevalence results of samples from 2011 [20], shedding light on the LB seroprevalence in Europe 50 years ago.

Methods

Study samples

The Finnish Mobile Clinic Health Survey (FMC) was a cross-sectional health survey of over 50000 voluntary Finnish participants aged ≥15, where data were collected in 31 municipalities in different parts of Finland during the years 1966–1972 [28]. As the study was conducted in the time preceding the current legislation on ethics in medical research, agreement to participation in the study was taken as giving informed consent [28]. The survey data included results of a wide-ranging health questionnaire, physical examinations, x-ray examinations, and electrocardiograms. Importantly, sera of the study participants were also collected.

We used a subset of 994 serum samples of the FMC survey collected throughout the year from 546 men and 448 women aged 15–86 years from 24 locations in different parts of Finland during the years 1968–1972. Although the sample panel is not fully representative of the general population in 1968–72, it represents the original FMC study population in the distribution of the regions, sexes, and age groups, except that the age groups >40 years are somewhat overrepresented. We were unable to use sampling weights for adjusting the analyses.

The volume of the samples was about 5 mL, and the samples were stored at −20°C. Before analyses, samples were thawed at room temperature, visually examined, and vortexed as all samples contained some precipitate. For serum quality check, the IgG antibody level in 41 randomly selected samples was measured using an in-house ELISA towards varicella zoster virus (VZV) [29]. All but three samples contained detectable levels of VZV IgG antibodies, reflecting the VZV antibody levels in the population today.

Serological testing algorithm

All serum samples ($n = 994$) were screened for IgG antibodies by an in-house *Borrelia* whole-cell sonicate (WCS) ELISA. The screening-positive serum samples (WCS IgG result ≥20 enzyme immunoassay units (EIU); $n = 358$) were further analysed by C6 Lyme ELISA test (Immunetics, Boston, USA). Sera with a positive result (Lyme index (LI) ≥0.9; $n = 205$), or sera with LI < 0.9, but with WCS IgG result ≥40 EIU ($n = 7$) were further analysed with recomBead IgG 2.0 (Mikrogen, Neuried, Germany) (Fig. 1). The serology procedure was identical to that described in our 2011 study [20].

Borrelia whole-cell sonicate IgG ELISA

The IgG antibodies in serum samples towards *Borrelia* WCS were measured as described previously [20]. Briefly, serum samples (1:100) were allowed to adhere to wells coated with sonicate of *B. burgdorferi* ss B31. The IgG levels were detected with alkaline phosphate-conjugated goat anti-human IgG secondary antibody (1:20000; Calbiochem, Darmstadt, Germany) and p-nitrophenyl phosphate substrate (Reagena, Toivala, Finland), and the reaction was stopped with 1 M sodium hydroxide. The absorbance was measured at 405 nm.

C6 Lyme ELISA and recomBead IgG 2.0

The antibodies in the screening-positive samples towards the synthetic C6 peptide were measured according to protocol of the manufacturer and as described previously [20].The samples that were C6-positive (LI ≥ 0.9) or C6-negative (LI < 0.9, but with WCS IgG result ≥40 EIU) were analysed with recomBead IgG 2.0 (Mikrogen) as described previously [20]. Briefly, magnetic polystyrene beads (MagPlex beads) coated with p100, VlsE, p58, p39, OspA and OspC of either *B. burgdorferi* ss, *B. afzelii*, or *B. garinii*, and p18 of *B. burgdorferi* ss, *B. afzelii*, *B. bavariensis*, *B. garinii*, and *B. spielmanii*, MAGPIX System with Luminex® xPONENT software, and Mikrogen recomQuant evaluation were used to determine the IgG levels. The serum samples were interpreted as positive (test result >3 points), borderline (3 points) or negative (0–2 points). The serum samples with a borderline test result were reported as positive.

For an additional sample quality check, 50 samples negative in the *Borrelia* WCS ELISA test were analysed with recomBead IgG 2.0 as described above. All samples remained clear negative.

Statistical analysis

The laboratory results were combined with the general questionnaire data. We calculated the seroprevalence estimate for the whole country and stratified for sex, age groups, region, employment status, field of employment, and exercise activities. Further, self-reported diseases, symptoms, and general health-related questions were selected from the FMC health questionnaire in order to determine whether LB seroprevalence was associated with any conditions indicative of disseminated LB, as LB was an unknown disease during the time of the FMC study. The selected diseases included cardiovascular, rheumatic, skin and neurological conditions, and non-specific symptoms suggestive of unhealthiness, such as the self-perception of feeling unhealthy, headache, and the intake of analgesics.

We used single variable logistic regressions to assess the association of each risk factor with LB seropositivity separately; we then included all variables with a p-value <0.20 in a multivariable model. The association of LB with self-reported diseases, symptoms and perception of health was analysed with univariate and single

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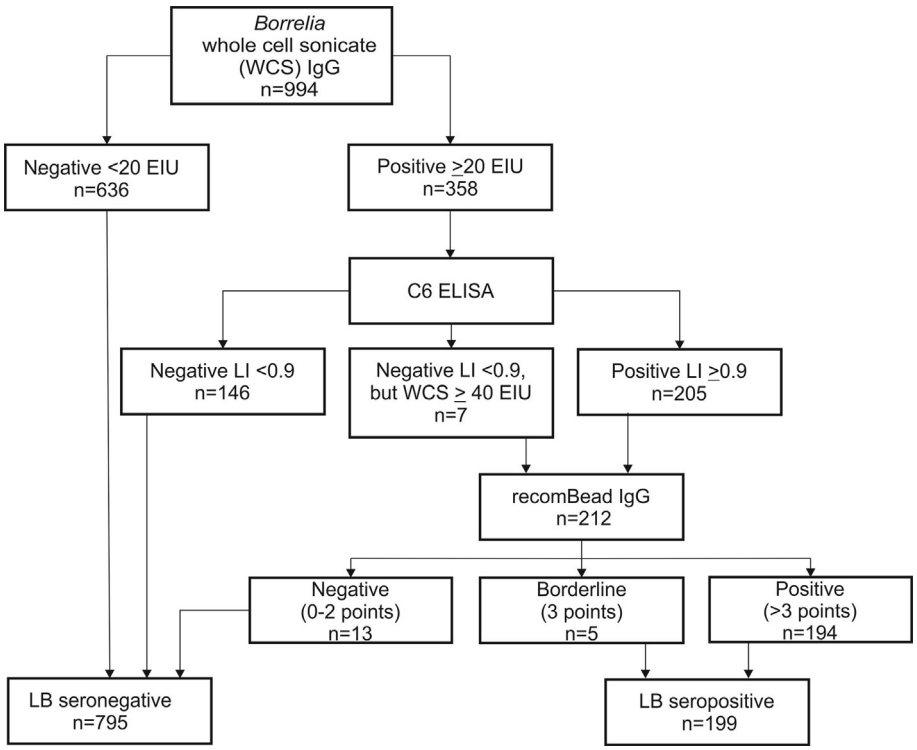

Fig. 1. Schematic overview of diagnostic assay algorithm; 199 of 994 serum samples were determined seropositive for Lyme borreliosis (LB) by *Borrelia* whole-cell sonicate (WCS) screening test and two confirmatory tests (C6 Lyme ELISA test and recomBead IgG 2.0 assay).

variable logistic regression adjusted for age groups, sex, and region. The results were displayed using odds ratios (ORs) and respective 95% confidence intervals (95%CI). The statistical significance level was considered at the 5% level. Statistical analysis was conducted using SPSS Statistics Software version 25.0 (IBM Corporation, Armonk, NY, USA).

Results

The median age of the study participants was 57 (range 15–86) years, and 448 of 994 (45.1%) were female (Table 1). After screening the 994 samples with *Borrelia* WCS ELISA, 358 (36.0%) were analysed with C6 peptide ELISA (Fig. 1); 205 (20.6%) C6-antibody-positive samples and seven (0.7%) C6-antibody-negative samples, but with *Borrelia* WCS IgG result ≥40 EIU, were further analysed by recomBead IgG 2.0 Supplementary Material Fig. S1. After the bead immunoassay, 13 sera were negative (1.3%), five (0.5%) were borderline and 194 (19.5%) were positive. In total, 199 of 994 (20.0%) sera were tested as *Borrelia* IgG-positive, resulting in an unweighted seroprevalence of 20.0% (95%CI: 17.6–22.6). The factors associated with LB seroprevalence are shown in Table 1. The seroprevalence among males (21.8%) was slightly higher than in females (17.9%) without statistical significance. The LB seroprevalence significantly increased with age ($p < 0.001$), was more common in persons currently unemployed than in employed persons (24.0% versus 17.5%), and among residents in south (40.3%) or central Finland (30.7%). Further, after adjustment for sex, age groups, and region, LB seropositivity showed a statistically significant inverse association with the self-reported perception of feeling healthy (adjusted OR: 0.6 (95%CI: 0.4–0.9), p 0.016); Supplementary Material Table S1). Of the self-reported diseases, previous heart failure and current heart valvular disease were statistically significantly associated with LB seropositivity (adjusted OR: 2.0 (95%CI: 1.1–3.7), p 0.035 and adjusted OR: 3.8 (95%CI: 1.3–11.2), p 0.015, respectively; Supplementary Material Table S1).

Discussion

The disease burden of LB in the late 19th and early 20th centuries—before the identification of the causative *Borrelia* spirochetes was known and predating LB seroprevalence studies—only date back to the early 1990s. To our knowledge, this is the first study investigating the LB seroprevalence in a European population in the 1960s and 1970s. There are case reports of certain skin lesions and neurological manifestations occurring after a tick bite prior to the time of *Borrelia* identification [22,23]. However, the ticks were neither recognized as carriers of *Borrelia* bacteria, nor were the patients systematically treated with antibiotics, although penicillin, for example, was available already in the 1940s. At the same time, the European economy, especially that in Northern Europe, was heavily based on agriculture and forestry, two occupations that are associated with the typical habitats of ticks [1]. Therefore, the presence of LB in Europe in the late 19th and early 20th centuries is to be expected.

We report the LB seroprevalence in Finland in 1968–72 to be 20.0%. The overall seroprevalence in the 1970s was five times higher than in our recent seroprevalence study from 2011 with a seroprevalence estimate of 3.9% [20]. The results of these two seroprevalence studies are not fully comparable, as only adults >29 years were included in the study from 2011 [20]. However, given the clear age-dependent increase in LB seroprevalence in both studies and the marked difference in the estimated seroprevalences, the sampling differences most likely do not account for the observed difference.

Hence, the considerable difference could partly be explained by the fact that Finland in the 1960s and 1970s was still largely an agrarian society, in contrast to the service-based society of the

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Table 1
Univariate and multivariable analysis of factors associated with Lyme borreliosis (LB) seropositivity in Finland in the years 1968–72

	No. persons IgG-positive/no. tested	LB seroprevalence (95%CI)	Univariate analysis		Multivariable analysis	
			OR (95%CI)	p	OR (95%CI)	p
Sex:						
Female	80/448	17.9 (14.5–21.6)	1	0.123	1	0.130
Male	119/546	21.8 (18.5–25.4)	1.3 (0.9–1.8)		1.3 (0.9–1.9)	
Age (years):						
15–29	2/44	4.5 (1.0–13.8)	1	0.000	1	0.013
30–39	6/68	8.8 (3.8–17.3)	2.0 (0.4–10.6)		1.3 (0.3–7.1)	
40–49	26/186	14.0 (9.6–19.5)	3.4 (0.8–15.0)		2.1 (0.5–9.64)	
50–59	68/291	23.4 (18.8–28.5)	6.4 (1.5–27.1)		4.3 (1.0–19.0)	
60–69	58/253	22.9 (18.1–28.4)	6.3 (1.5–26.6)		3.7 (0.8–16.2)	
>70	39/152	25.7 (19.2–33.0)	7.3 (1.7–31.4)		3.6 (0.8–16.5)	
Field of work:						
Agriculture and Forestry	59/283	20.8 (16.4–25.9)	1	0.547	not in the model	
Industry and Mining etc.	66/293	22.5 (18.0–27.6)	1.1 (0.7–1.6)			
Office etc.	4/35	11.4 (4.0–24.9)	0.5 (0.2–1.4)			
Transportation and logistics	10/40	25.0 (13.6–39.8)	1.3 (0.6–2.7)			
Commerce	6/52	11.5 (5.0–22.2)	0.5 (0.2–1.2)			
Service	15/76	19.7 (12.0–29.7)	0.9 (0.5–1.8)			
Business management and teachers	11/74	14.9 (8.2–24.2)	0.7 (0.3–1.3)			
Housewives	16/88	18.2 (11.2–27.2)	0.8 (0.5–1.6)			
Students and schoolchildren	1/8	12.5 (1.4–45.4)	0.5 (0.1–4.5)			
Pensioners	11/45	24.4 (13.7–38.2)	1.2 (0.6–2.6)			
Current employment status:						
Not working	92/383	24.0 (19.9–28.5)	1	0.013	1	0.046
Working	107/611	17.5 (14.7–20.7)	0.7 (0.5–0.8)		0.7 (0.5–1.0)	
Sport/exercise activities:						
No	65/365	17.8 (14.1–22.0)	1	0.169	not in the model	
Yes	134/625	21.4 (18.4–24.8)	1.3 (0.9–1.8)			
Less than once a week	18/97	18.6 (11.8–27.2)	1.1 (0.6–1.9)	0.350		
Once a week	34/170	20.0 (14.5–26.5)	1.2 (0.7–1.8)			
Daily	75/323	23.2 (18.9–28.0)	1.4 (1.0–2.0)			
Large areas of Finland:						
Southwest	21/182	11.5 (7.5–16.8)	1	0.000	1	0.000
South	91/226	40.3 (34.0–46.7)	5.2 (3.1–8.8)		4.7 (2.7–8.0)	
Central	27/88	30.7 (21.8–40.8)	3.4 (1.8–6.4)		3.5 (1.8–6.8)	
West	5/82	6.1 (2.4–12.8)	0.5 (0.2–1.4)		0.4 (0.2–1.1)	
East	42/254	16.5 (12.4–21.5)	1.5 (0.9–2.7)		1.4 (0.8–2.5)	
North	13/162	8.0 (4.6–13.0)	0.7 (0.3–1.4)		0.6 (0.3–1.3)	

OR (95%CI), odds ratio (95% confidence interval).

present day. As can be seen in the background data of the study subjects, approximately one third of the study participants worked in the field of agriculture or forestry. In contrast, in 2011, only 24 of 2000 study participants were agricultural entrepreneurs [20]. Interestingly, the high LB seroprevalence in the general population in Finland 50 years ago appears to be similar to the seroprevalence observed in farmers, forestry workers, orienteers, and in residents of LB-endemic regions of the present day [13–19]. However, in the 1960s and 1970s, the LB seroprevalence among farmers and forestry workers was not statistically significantly higher than among study subjects working in any other field. In fact, the LB seroprevalence was higher among the study participants who were not employed at the time of recruitment to the study. The unemployed study participants included housewives and students, who possibly spent more time gardening, berry picking and doing sport activities, which could have led to more frequent encounters with ticks.

The higher seroprevalence in males and in older age groups parallels the trend observed in other studies [9–11,20]. Surprisingly, the seroprevalence in central and south Finland was higher than in southwest Finland, which today is a region with probably the highest tick densities and high LB incidence rates in Finland [20]. Another curiosity is the detection of 13 seropositive study participants in northern Finland, which is a region with low LB annual incidence at present [20]. However, an obvious explanation to the observed seropositive subjects in northern Finland is that people were travelling and moving around the country in the 1960s and1970s, as they are doing today, and we do not know the exact location of the tick exposure of the study subjects.

Finally, in 128 of 199 positive samples, the IgG antibodies towards the p18 of *B. afzelii*, an antigen associated with late disseminated LB [4], were over the detection limit in the recomBead IgG 2.0 bead immunoassay Supplementary Material Fig. S1, which is in contrast to the results of the 2011 samples, where no samples yielded the 'over' result with this antigen. This observation tempts us to speculate that the study subjects possibly had an ongoing disseminated LB, or that they had been exposed to *Borrelia* several times leading to a prominent immune response. Furthermore, without the understanding of the various presentations of LB that we have today, the participants were most likely misdiagnosed with (for example) rheumatoid arthritis or Bell's palsy.

However, when we explored the self-reported diseases and symptoms, only non-LB-related heart failure and heart valvular disease were statistically significantly associated with LB seropositivity. As *Borrelia* usually causes atrioventricular nodal block [1], the aforementioned heart conditions were presumably not clinically significantly associated with LB. Interestingly, the LB-seropositive participants more frequently reported feeling unhealthy than the seronegative participants. Hence, this could suggest that the LB-seropositive participants experienced general symptoms relating to an ongoing LB infection. However, in contrast to the present results reflecting the situation in Finland half a century ago, in a recent study from Norway no subjective health complaints were associated with LB seropositivity [30]. Notably, we

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acknowledge that the analysis of the self-reported diseases, symptoms and perception of health is explorative, and we can only speculate on their association with LB seropositivity.

In summary, we demonstrate here that LB seroprevalence was considerably higher in Finland in the 1960s and 1970s than in 2011. Furthermore, older age, unemployment, and living in south and central Finland were risk factors associated with LB seropositivity. This study sheds light on LB seroprevalence in Europe half a century ago, and challenges the scale of the present-day scare around ticks and tick-borne diseases.

Transparency declaration

Authors declare no conflict of interest related to this article. This work was supported by a grant from the Jane and Aatos Erkko Foundation.

Authors' contributions

JH and JS designed the study and contributed equally to it. JC and JH did the laboratory analyses and interpretation of the results. JC, TD and JS analysed the data. JC and JH wrote the manuscript and produced the figures and tables. TD and JS gave constructive feedback on the manuscript.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.cmi.2019.10.003.

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