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A serosurvey examining exposure to *Borrelia burgdorferi sensu lato* and tick-borne encephalitis virus in Danish blood donors, August 2022

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ABSTRACT

Objectives: *Borrelia burgdorferi sensu lato* (Bbsl) and tick-borne encephalitis virus (TBEV) are tick-borne pathogens. This study aimed to investigate the seroprevalence of these pathogens in Danish blood donors.

Methods: A total of 1000 plasma samples equally distributed (n = 200) from all five Danish regions were analyzed. Commercially available enzyme-linked immunosorbent assays were used to screen the samples for immunoglobulin G antibodies against Bbsl and TBEV. The samples positive for antibodies against TBEV were further examined with a commercially available enzyme-linked immunosorbent assay and a Luminex-based TBEV suspension multiplex immunoassay for specific antibodies against non-structural protein 1 (NS1) antigen suggestive of previous infection.

Results: A total of 62 samples tested positive for immunoglobulin G antibodies against Bbsl. A total of 40 samples were positive or borderline for antibodies against TBEV, indicating potential infection or vaccination. Of these, one had antibodies against NS1, indicating past infection. The seroprevalence of Bbsl was 6.2% (95% confidence interval 4.8-7.8), with equal seroprevalence in all five regions. The seroprevalence of TBEV was 0.1% (95% confidence interval 0.01-0.62%).

Conclusions: The seroprevalence of Bbsl was similar throughout the country and corresponds well with previous studies. The seroprevalence of TBEV NS1 was low, which is in line with a low number of reported tick-borne encephalitis cases in Denmark. The NS1 positive sample was from the Capital Region, an endemic TBEV area.

Introduction

Lyme borreliosis, caused by spirochetes in the *Borrelia burgdorferi sensu lato* (Bbsl.) complex, is the most common tick-transmitted infection in Europe. Tick-borne encephalitis (TBE), caused by the flavivirus TBE virus (TBEV), is the most common tick-transmitted viral infection in Europe, with 24 countries reporting 3734 confirmed cases in 2020 and an increase in notification rate since 2018 [1].

Ixodes ricinus ticks infected with *B. burgdorferi* s.l. have been reported throughout Denmark, with infection rates between 3.5% and 23.5% detected in the nymphal stage, the stage responsible for most human transmissions [2]. Despite *B. burgdorferi* s.l. being widespread in Danish ticks,

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little is known about the human infection rates of Lyme borreliosis because only Lyme neuroborreliosis is notifiable in Denmark, with an annual incidence of 2.6 per 100,000 [3].

TBEV circulates between mammals and ticks in geographically restricted "microfoci," sometimes only covering some 50 × 50 m, that are highly dependent on biotic and abiotic factors which affect all components of TBEV transmission [4]. Hence, detecting the virus in ticks may be challenging, and, in some areas, the virus may disappear from the environment within years [5]. Ticks infected with TBEV have been reported from several localities in Denmark at estimated prevalence rates between 0.2% and 8% [6–8], with the highest prevalence of 8% found in the middle of a TBEV endemic microfocus [8]. The island of Bornholm is known to be endemic to TBEV since the 1950s, and, since 2019, an area in Northern Zealand is considered endemic as well. Annually, five to 10 TBE cases are reported in Denmark [9].

A few studies examining antibodies against *Borrelia burgdorferi* s.l. and TBEV in Danish regions and in a certain high-risk group have been published [10,11]; however, to the best of our knowledge, no studies have examined the seroprevalence on a national level. Furthermore, data on the incidence of these infections are lacking because only Lyme neuroborreliosis is notifiable and TBE is not notifiable in Denmark. Because insufficient knowledge about the actual distribution of TBEV in ticks can potentially lead to underdiagnosis of TBE, it is important to elucidate the level of exposure in a Danish setting.

This study aimed to perform a serosurvey to assess human exposure to *B. burgdorferi* s.l. and TBEV in all five Danish administrative regions, thereby investigating the potential occurrence of these tick-transmitted infections.

Materials and methods

Study population

Plasma samples from 1000 blood donors were obtained in August 2022 from residual material after blood donation; 200 samples were taken from each of the five Danish regions: Capital Region, Region Zealand, North Denmark Region, Central Denmark Region, and Region of Southern Denmark. For analyses, five age groups were defined: age 17-30 (n = 211), 31-40 (n = 177), 41-50 (n = 233), 51-60 (n = 239), and 61-71 years (n = 140) (Supplementary Table S1).

Serology

Commercially available enzyme-linked immunosorbent assays (ELISAs) were used to screen for immunoglobulin (Ig) G antibodies against *B. burgdorferi* s.l. (Virion-Serion, Germany) and TBEV (Euroimmun, Germany) in all plasma samples, according to the manufacturer's instructions.

To discriminate between IgG antibodies against TBEV elicited by past infection and those induced by vaccination against TBEV or cross-reactivity with other flaviviruses, samples found positive and borderline by the Euroimmun TBEV ELISA kit were further analyzed to detect antibodies against non-structural protein 1 (NS1) by a commercially available ELISA and a Luminex-based TBEV suspension multiplex immunoassay (SMIA) [12]. The human TBEV NS1 IgG ELISA (Alpha Diagnostic International, USA) was performed according to the manufacturer's recommendations and instructions diluting samples 1:200. Cut-off values were calculated according to the antibody activity threshold index. The Luminex-based TBEV SMIA based on the NS1 antigen and whole virus was performed at the Zoonosis Science Center at the Department of Medical Biochemistry and Microbiology, Uppsala University [12].

Statistical analyses

Seroprevalence of *B. burgdorferi* s.l. and TBEV was estimated as the number of positive samples out of the total number of samples in the group being examined. Borderline results were considered negative in the data analyses. The *Z* test and Fischer's exact test were used to compare two proportions. Univariable and multivariable logistic regression were used to examine the effect of the age and sex (independent variables) on seropositivity (dependent variable). The two-way interaction between age and sex was tested. A $P < 0.05$ was considered significant. Statistical analyses were performed in GraphPad Prism (Version 9.5.1 [733], January 2023).

Results

Study population

A total of 1000 blood donor samples were analyzed for IgG antibodies against *B. burgdorferi* s.l. and TBEV (Figure 1). The median age of the entire study population was 46 years (interquartile range 32-56 years) and the female-to-male ratio was 0.9:1, with a similar median age for all five administrative regions of Denmark (Table 1).

Antibodies against *B. burgdorferi* s.l. by ELISA

A total of 62 of 1000 samples (6.2%, 95% confidence interval [CI]: 4.8-7.8%) were positive for IgG antibodies against *B. burgdorferi* s.l. There were significantly more positive males (44 of 539; 8.2%) than females (18 of 461; 3.9%) ($P = 0.005$). There was no significant difference

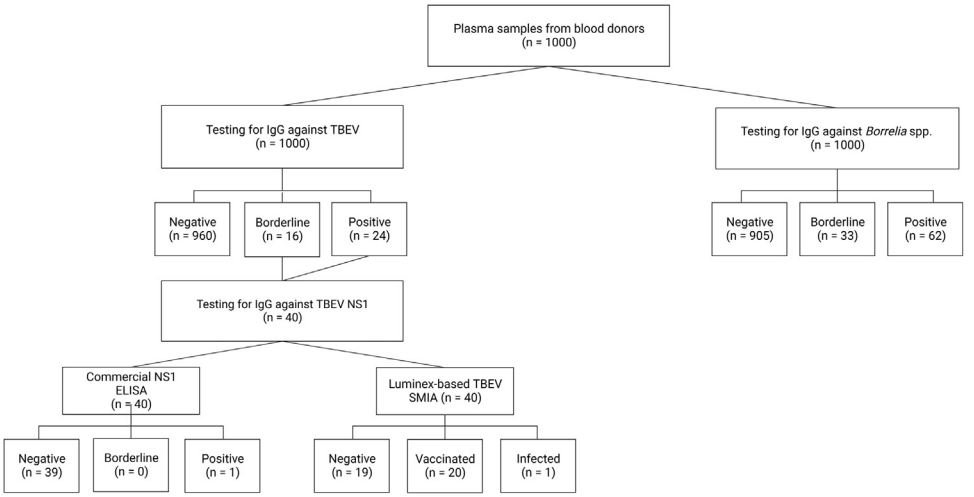

Figure 1. A flowchart of the analyses performed on blood donor samples for detection of antibodies against *Borrelia burgdorferi sensu lato*, TBEV, and NS1, Denmark (n = 1000). ELISA, enzyme-linked immunosorbent assays; Ig, immunoglobulin; NS1, non-structural protein 1; SMIA, suspension multiplex immunoassay; TBEV, tick-borne encephalitis virus.

Table 1
Clinical characteristics of the blood donors across the five Danish administrative regions, Denmark (n = 1000).

	Female		Male		All	
Region	n	Median age, years (IQR)	n	Median age, years (IQR)	Median age, years (IQR)	Female: Male
Capital Region	95	46 (30–53)	105	44 (32–55)	44.5 (31–54)	0.9:1
Central Denmark Region	104	49 (32–58)	96	44.5 (30–57)	46 (32–58)	1.1:1
North Denmark Region	100	42.5 (30–55)	100	46 (34–56)	44.5 (31–55)	1:1
Region of Southern Denmark	77	44 (29–53)	123	45 (31–56)	44.5 (31–55)	0.6:1
Region Zealand	85	47 (34–57)	115	48 (38–57)	48 (37–57)	0.7:1
All regions	461	45 (35–53)	539	46 (37–55)	46 (32–56)	0.9:1

IQR, interquartile range.

Table 2
Borrelia burgdorferi sensu lato immunoglobulin G seroprevalence in females and males grouped by administrative region, Denmark (n = 1000).

	Female				Male				
Region	Total (n)	Positive (n)	Positive (%)	95% CI	Total (n)	Positive (n)	Positive (%)	95% CI	*P*-value
Capital Region	95	3	3.2	0.7-9.3	105	10	9.5	5.1-16.8	0.087
Central Denmark Region	104	4	3.9	1.2-9.8	96	6	6.3	2.6-13.2	0.525
North Denmark Region	100	5	5	1.9-11.5	100	11	11	6.1-18.8	0.191
Region of Southern Denmark	77	4	5.2	1.6-13	123	12	9.8	5.5-16.4	0.295
Region Zealand	85	2	2.4	0.1-8.7	115	5	4.4	1.6-10	0.701

CI, confidence interval.

in overall *B. burgdorferi* s.l. seroprevalence between regions ($P = 0.262$) (Figure 2) nor between female and male within each region (Table 2). However, because the minimum regional seroprevalence was 3.5% and the maximum was 8% and the seroprevalence in females being consistently lower than in males in all regions, these results may be because of the lack of statistical power or coincidence. Seroprevalence increased with age (Supplementary Table S2). Significant interactions between sex (female as the reference) and age were found in age group 31-40 (odds ratio [OR]: 22, 95% CI: 1.4-953, $P = 0.049$), 41-50 (OR: 15, 95% CI: 1.4-350, $P = 0.043$), and 51-60 years (OR: 12, 95% CI: 1.3-265, $P = 0.048$) compared with age group 17-30 years.

Antibodies against whole virus tick-borne encephalitis virus

A total of 24 of 1000 samples (2.4%, 95% CI 1.6-3.5%) were positive and 16 of 1000 (1.6%, 95% CI 0.9-2.6%) were borderline using the Euroimmun ELISA assay (Figure 1). No statistical analyses were performed due to the lack of statistical power.

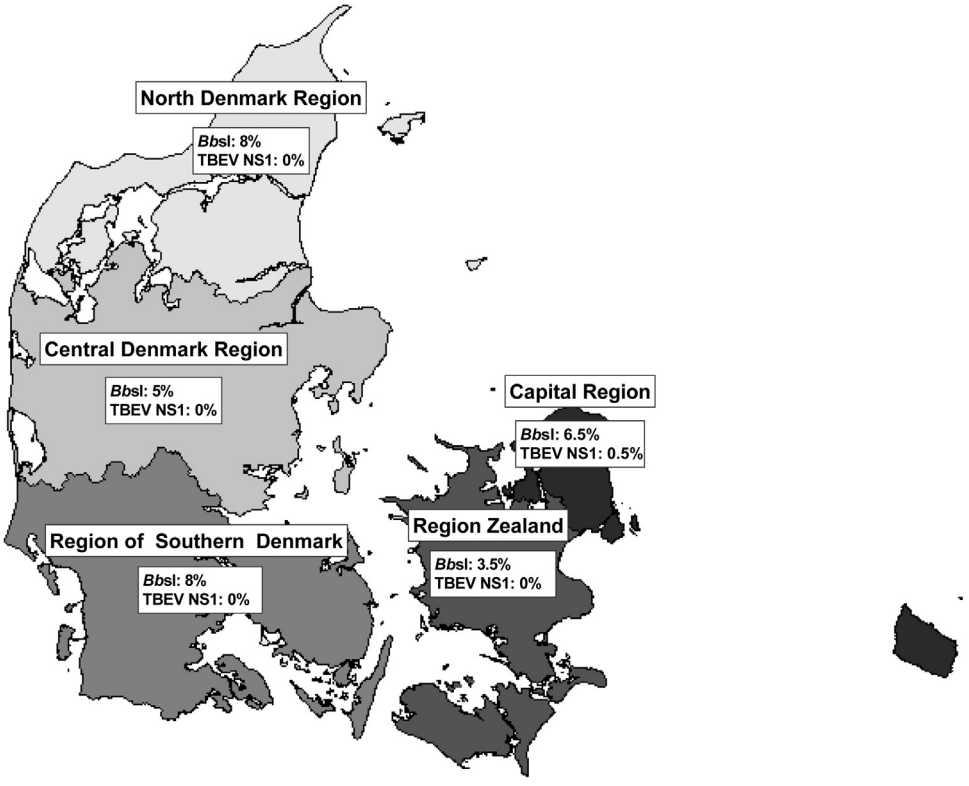

Figure 2. IgG seroprevalences of Bbsl and TBEV NS1, all five administrative regions, Denmark (n = 1000).
Bbsl, *Borrelia burgdorferi sensu lato*; TBEV NS1, tick-borne encephalitis virus non-structural protein 1.

Table 3
Antibodies detected against TBEV NS1 and TBEV WV in SMIA and ELISA assays. Samples positive and borderline in the Euroimmun assay were subsequently analyzed by SMIA and NS1 ELISA (n = 40).

	SMIA		ELISA	
	NS1[a] (Infected)	WV[b] (Vaccinated)	Alpha diagnostic international NS1[c]	Euroimmun WV[d] (Borderline and positive)
All regions	**1**	**20**	**1**	**40**
Capital Region	1	8	1	11
Central Denmark Region		4		10
North Denmark Region		3		6
Region of Southern Denmark		2		6
Region Zealand		3		7

ELISA: enzyme-linked immunosorbent assays; NS1: non-structural protein 1; SMIA: suspension multiplex immunoassay; TBEV: tick borne encephalitis virus; WV: whole virus.
[a] European;
[b] Strain Moscow B-4;
[c] Neudoerf sequence strain;
[d] Strain K23.

In total, 20 samples were considered positive owing to vaccination in the whole virus Luminex-based TBEV SMIA assay (Table 3).

Antibodies against TBEV NS1 antigen

One sample was positive in the Alpha Diagnostic International NS1 ELISA and the same sample was positive in the NS1 Luminex-based TBEV SMIA assay, indicating past infection. The seroprevalence of TBEV was one per 1000 (0.1% [95% CI 0.01-0.62%]) (Figure 2).

Discussion

To the best of our knowledge, this is the first study to examine IgG antibodies against *B. burgdorferi* s.l. and TBEV nationwide in Danish blood donors. IgG antibodies against *B.* burgdorferi s.l. were detected in 6.2% (62 of 1,000) of blood donors and were equally distributed in the five Danish regions. Significantly fewer females (3.9%, 18 of 461) than males (8.2%, 44 of 539) had antibodies against *B. burgdorferi* s.l., and an increasing seropositivity rate was observed with increasing age. Antibodies against the TBEV NS1 antigen, indicative of past TBEV infection, were only detected in one (0.1%) sample from the Capital Region, which is known to have endemic microfoci of TBEV [13]. A total of 20 samples were positive for antibodies against TBEV but not TBEV NS1, which could be indicative of TBE vaccination.

Our data show that *B. burgdorferi* s.l. seroprevalence is similar in all five Danish regions, which is consistent with ticks being present and infected with the bacteria to a similar extent throughout Denmark [2]. A national seroprevalence of 6.2% is comparable with other Danish studies analyzing sporadic samples collected between 2015 and 2019 [14]. Seroprevalences in neighboring countries have ranged from 3.4% in Norway [15] to 7.4% in Sweden [16] and 9.4% in Germany [17]. However, seroprevalences of 16.9% in Germany [18] and 19.7% in Sweden [19] have been reported from highly endemic areas.

In line with our results, several studies have reported a significantly higher seroprevalence of *B. burgdorferi* s.l. in males than in females [15,17,20], although contradicting results have been reported [21]. Notably, the difference in the seroprevalence in our study was not due to older age in the males compared with females. The higher exposure rate among males corresponds well with a generally higher incidence of Lyme neuroborreliosis among males [3]. Our findings indicate that *B. burgdorferi* s.l. seroprevalence increases with age, which is consistent with a reported increase of seroprevalence of *B. burgdorferi* s.l. with increasing age in both males and females in other seroprevalence studies [11,17,20]. This pattern likely reflects *B. burgdorferi* s.l. antibody persistence over time [22].

The overall prevalence of TBEV in ticks in Scandinavia is 0.28% but with large local differences in tick infection [23] and human incidences. Sweden reported annual incidences between 0.16-5.11 per 100,000 from 1956 to 2022 [24] and Norway reported 0-1.6 per 100,000 annually from 1994 to 2022, including some cases acquired abroad [25]. In Denmark, annual incidences between 0-0.15 per 100,000 TBE cases acquired within the country were reported between 1994 and 2022, corresponding to four to five cases annually [13].

It has been estimated that 75-98% of TBEV infections in Europe are subclinical [26]. A recent German study estimated a significantly higher incidence of subclinical infections (>250 per 100,000 per year) compared with reported cases (4.7 per 100,000 per year) [26]. We detected a national TBEV seroprevalence of 0.1%. A regional seroprevalence of 0.5% was observed in the Capital Region, a TBEV endemic area in Denmark, with the majority of Danish TBE cases being reported from the same region. Comparing this regional seroprevalence with reports from other countries examining healthy blood donors from high-endemic areas, the seroprevalence is comparable with a Norwegian study reporting a seroprevalence of 0.4% [27]. Seroprevalences as high as 5.3% in Sweden [28], 5.6% in Germany [26], and 7% in Switzerland [29] have also been reported. The German and Swiss studies reported significant increases in seroprevalence among blood donors compared with studies 40-50 years ago.

The samples positive using Euroimmun ELISA but negative using the NS1 assays likely mirror TBE vaccination status or cross-reactivity with antibodies against other flaviviruses. Although insignificant, most samples indicative of antibodies against TBEV owing to vaccination were collected from the Capital Region, which includes well-known TBEV microfoci, such as Northern Zealand and Bornholm. A higher vaccination rate in these regions is confirmed in a recent report by the Danish Public Health Agency Statens Serum Institut, Denmark [30].

Limitations

The vaccination status of the blood donors was unknown. We did not differentiate in sample size from each of the five regions, although the five Danish regions vary greatly in population size from 590,000 inhabitants in the North Denmark Region to approximately two million in the Capital Region. The occupation and area of residence of the blood donors were unknown. Blood donors are generally healthy and more physically active than non-donors [31]. Whether this physical activity includes more activity in nature, however, is unknown. The seroprevalence detected in this study may, therefore, be different from the general population. Furthermore, seroprevalence represents the cumulative exposure and not the incidence of new disease.

Conclusion

Our results contribute to the knowledge of the possible extent of *B. burgdorferi* s.l. and TBEV infections in Denmark. We found that IgG

antibodies against *B. burgdorferi* s.l. (6.2%) were more prevalent than IgG antibodies against TBEV (0.1%), which is consistent with the prevalence of these pathogens detected in Danish ticks. The blood donors in this study were likely equally exposed to ticks in all Danish regions based on the *B. burgdorferi* seroprevalence being similar in all regions but we only detected antibodies against TBEV in the Capital Region. Finally, we detected an increasing seroprevalence with increasing age, which may be indicative of antibody persistence.

Declarations of competing interest

None of the authors declare they have any financial conflicts of interests. Outside the present work, AML discloses speaker honoraria and advisory board activities from Gilead, ViiV/GSK, and Pfizer and a grant from The Lundbeck Foundation (R366-2021-127). The funding bodies had no role in writing the manuscript.

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Ethical statement

The study was approved by the Danish Data Protection Agency (Approval no. P-2023-173). The authors did not need ethical committee approval because they included anonymized biological samples collected according to the law at blood bank collection sites.

Author contributions

The first draft of the manuscript was written by Hansen MF and Gynthersen RMM. Ocias LF contributed with study conception and design and repeated revisions of the manuscript. Sørensen CA performed the ELISA. Harritshøj L, Erikstrup C, Holm DK, Sækmose SG, and Aagaard B contributed with blood donor samples. Kolstad L, Hoffman T, and Lindkvist Å contributed with performing the Luminex-based TBEV SMIA. Mens H contributed with conception and design. Conception, study design, supervision, and repeated revisions of the manuscript were performed by Lebech AM and Krogfelt KA. All authors critically revised, commented on, and approved the final manuscript.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.ijregi.2024.100414.

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