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Prevalence of *Borrelia burgdorferi* sensu lato infection in the Czech Republic

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ABSTRACT

Introduction: Lyme borreliosis (LB), an infection caused by *Borrelia burgdorferi* sensu lato (Bbsl), is the most common tick-borne disease in Europe. To further characterize the LB burden in the Czech Republic, we conducted a seroprevalence study and estimated the incidence of symptomatic Bbsl infections.
Methods: Anti-Bbsl IgM and IgG antibodies were detected in sera collected from the adult population in 2011 −2012 by enzyme-linked immunosorbent assay and immunoblot tests at the National Reference Laboratory. The incidence of symptomatic Bbsl infections was estimated from the seroprevalence results and the symptomatic proportion and duration of persistence of anti-Bbsl IgG antibodies in Bbsl-infected individuals. Surveillance under-detection of symptomatic Bbsl infections was estimated by comparing surveillance-reported and seroprevalence-based incidence.
Results: Samples from 1996 adults were tested; the median age (range) was 45 (18 −87) years; 1037 (52.0 %) were female. The prevalence (with 95 % confidence interval) of anti-Bbsl IgG, and IgM and/or IgG (IgM/IgG) antibodies was 6.3 % (5.3 −7.5 %), and 9.5 % (8.3 −10.9 %), respectively. The IgM/IgG prevalence was 7.8 % (6.5 −9.2 %) in Bohemia and 15.3 % (12.2 −19.0 %) in Moravia. There were an estimated 30,563 (26,550 −34,962) symptomatic incident Bbsl infections in adults in the Czech Republic in 2012, for an incidence of 352.2 (306.0 −402.9) symptomatic Bbsl infections per 100,000 adults per year. There were an estimated 11 (10 −13) symptomatic Bbsl infections for each surveillance-reported LB case in the Czech Republic in 2012.
Conclusions: There is high incidence of symptomatic Bbsl infections in the Czech Republic, particularly in Moravia. Interventions are needed to address the substantial burden of LB in the Czech Republic.

1. Introduction

Lyme borreliosis (LB), an infection caused by *Borrelia burgdorferi* sensu lato (Bbsl), is the most common tick-borne disease in Europe (Stanek et al., 2012; van den Wijngaard et al., 2017). Humans become infected with Bbsl via the bite of an infected *Ixodes* tick (Stanek et al.,

Abbreviations: Bp, *Bordetella pertussis*; Bbsl, *Borrelia burgdorferi* sensu lato; CI, confidence interval; ELISA, enzyme-linked immunosorbent assay; EM, erythema migrans; ECDC, European Centre of Disease Prevention and Control; IgG, immunoglobulin G; IgM, immunoglobulin M; LB, Lyme borreliosis; LNB, Lyme neuroborreliosis; NPHI, National Public Health Institute; NRL, National Reference Laboratory; PPY, population per year.

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2012). The most common clinical manifestation of LB is a localized skin disease called erythema migrans (EM), but Bbsl infections can disseminate and result in Lyme neuroborreliosis (LNB), arthritis, or carditis (Cardenas-de la Garza et al., 2019).

LB is endemic in many countries in Europe (Nagarajan et al., 2023) including the Czech Republic, which had a population 10.9 million in 2023. The Czech Republic and more than twenty other European countries conduct public health surveillance for LB and publish surveillance data online (Nagarajan et al., 2023). It is estimated that at least 202 million (24 %) of the 844 million persons in Europe reside in a high LB incidence area, defined as an area with an incidence of more than 10 surveillance-reported LB cases per 100,000 population per year (PPY) (Burn et al., 2023). In countries with comprehensive LB surveillance systems, approximately 2 −4 % of surveillance-reported LB cases are LNB (Brestrich et al., 2024; Geebelen et al., 2019; Hofhuis et al., 2015). The European Centre of Disease Prevention and Control (ECDC) began LNB surveillance in 2018 (Hy and Muhhamad, 2018); the most recent ECDC LNB surveillance data online is from 2021. Although the population in the Czech Republic is 3 % of the population of the twenty-five countries reporting surveillance data to ECDC, the Czech Republic reported 36 % (1303/3606) of the total LNB cases reported in 2019 −2021 (https://www.ecdc.europa.eu/en/surveillance-atlas-infectious-diseases).

LB has been a statutorily notifiable disease in the Czech Republic since the mid-1980s (Act Number 258/2000) with LB surveillance conducted by the National Public Health Institute (NPHI) (Orlíková et al., 2022). Clinicians report all clinical forms of LB to authorities at one of the 14 public health regions who report to NPHI; annual surveillance reports are available at https://szu.cz/en/publications-data/infections-in-the-czech-republic/. There were 3270 surveillance-reported LB cases in 2023, resulting in an incidence of 30.2 per 100,000 PPY. Despite the comprehensive surveillance system in the Czech Republic, LB surveillance likely under-detects individuals with incident symptomatic Bbsl infection due to LB patients not seeking medical care, seeking medical care but not being diagnosed with LB, or being diagnosed with LB but not being reported to surveillance (Orlíková et al., 2022).

Seroprevalence studies provide estimates of the prevalence of people with anti-Bbsl antibodies, which indicates current or previous Bbsl infection, and therefore are a useful tool for understanding the population-based LB burden. To further characterize the LB burden in the Czech Republic, we conducted a seroprevalence study of the general adult population to determine the prevalence of anti-Bbsl antibodies, estimate the incidence of symptomatic Bbsl infection, and estimate the under-detection of symptomatic Bbsl infection by surveillance.

2. Methods

Aliquot sera from a national pertussis seroprevalence study (Chlibek et al., 2017), which collected blood samples from the general adult population from October 2011−December 2012, were retrieved for a LB seroprevalence study. Participants in the seroprevalence study were healthy adults (≥18 years-of-age) who were recruited on a "first-come, first-serve" basis via an advertising campaign in the media. Enrollment and blood collection occurred at three blood donation centers, two centers in Bohemia (Ceske Budejovice and Hradec Kralove) and one center in Moravia (Brno). The seroprevalence study enrolled participants in accordance with the age structure in the Czech Republic in 2012.

At the time of blood collection, participants provided written consent for enrollment and, in compliance with Czech Republic rules, approval for use of aliquot sera for future research purposes. After the blood was collected, sera were stored at the central blood donation center in Hradec Kralove at −20°C and transferred to the Czech National Reference Laboratory (NRL) for Pertussis and Diphtheria at NIPH for testing for anti-*Bordetella pertussis* (Bp) antibodies in 2012. Residual aliquot sera were stored at −20°C at the Hradec Kralove blood donation center until retrieval for the LB seroprevalence study. To assess the quality of sera storage, 50 selected sera samples were retrieved and retested for anti-Bp immunoglobulin G (IgG) antibodies in February 2024.

In March 2024, all aliquot sera were retrieved and transferred to the Czech NRL for Lyme Borreliosis at NIPH for anti-Bbsl antibody testing. Available information for sera was limited to date of the collection, blood donation center where blood was collected, and donor's age, sex, and postal code of residence at time of blood collection. The first digit of the postal code was used to define the residence location of the participants; 1 and 2: Central Bohemia (which includes Praha [Prague]); 3: Western/Southern Bohemia (Karlovy Vary, Plzen, and Ceske Budejovice); 4: Northern Bohemia (Usti nad Labem, and Liberec); 5 Eastern Bohemia/Western Moravia (Hradec Kralove, and Pardubice); 6 Southern Moravia (Brno), 7 Eastern/Northern Moravia (Olomouc, Zlin, Ostrava). Residence locations were then aligned to the 14 regions of the Czech Republic and consolidated into the areas of Bohemia (regions of Karlovy Vary, Plzen, Usti nad Labem, Southern Bohemia, Central Bohemia, Liberec, Vysocina, Pardubice, Hradec Kralove, and Praha), and Moravia (regions of Southern Moravia, Zlín, Olomouc and Moravian-Silesian).

At the NRL for Lyme borreliosis, a two-tier laboratory testing approach was used for the detection of anti-Bbsl antibodies. In the first-tier test, samples were tested by an enzyme-linked immunosorbent assay (ELISA) test (TestLine Clinical Diagnostics, Czech Republic) for immunoglobulin M (IgM) and IgG antibodies to *Borrelia* spp. using test procedures and interpretive criteria in accordance with the manufacturer's instructions. All sera with a positive or borderline (equivocal) result in the first-tier testing were tested with the second-tier confirmatory test using the Microblot-Array *Borrelia* IgM and IgG immunoblot test (TestLine Clinical Diagnostics, Czech Republic) which contained immunodominant recombinant *Borrelia* spp. specific antigens identical to IgM and IgG (VlsE, OspA, OspC, p41) from *B. afzelii*, *B. garinii, B. burgdorferi* sensu stricto, *B. spielmanii* and other recombinant *Borrelia* antigens (p83, p58, p39, OspB, OspE, p17 and NapA). The immunoblot test procedure and interpretation criteria were performed in accordance with the manufacturer's instructions and evaluation of the test results was performed using MBA Software (TestLine Clinical Diagnostics, Czech Republic).

In the base case analysis, the number of adults with an incident Bbsl infection in 2012, with 95 % confidence interval (CI), was estimated from the number of adults with anti-Bbsl IgM and/or IgG (IgM/IgG) antibodies from the seroprevalence study using the formula: I=P/D, where I was the number of incident Bbsl-infected adults, P was number of adults with anti-Bbsl IgM/IgG antibodies (with 95 % CI) from the seroprevalence study, and D was the median duration of detection of anti-Bbsl IgG antibodies. Similar calculations were conducted in the sensitivity analysis except P was the number of adults with anti-Bbsl IgG antibodies in the seroprevalence study. In the calculations, we used an estimate of the median duration of detection of anti-Bbsl IgG antibodies of 10 years (Angulo et al., 2024). We also used an estimate of the proportion of incident Bbsl-infected individuals with symptoms of 37 % (Angulo et al., 2024) to estimate the number (with 95 % CI) of symptomatic Bbsl-infected adults.

The estimated number of symptomatic Bbsl-infected adults and the adult population of the Czech Republic (8676,895), Bohemia (5681,725), and Moravia (2995,170) were used to estimate the incidence of symptomatic Bbsl infection in adults (i.e., number of symptomatic incident cases per 100,000 adults PPY) in 2012 by region (with 95 % CI). The estimated incidence (with 95 % CI) of symptomatic Bbsl infections in 2012 was then compared to the incidence of surveillance-reported LB cases in adults in 2012 to estimate the extent of under-detection of adult symptomatic LB cases by public health surveillance. Population estimates for the adult population were derived from the Czech Statistical Office and State Health Institute (https://csu.gov.cz/).

3. Results

In the initial assessment of the quality of serum storage, there was a high correlation between the anti-Bp IgG titers for the 50 samples tested in 2012 and 2024 (Pearson correlation coefficient 0.9448, p < .0001) (Supplementary Figure 1). Stored residual sera from 1996 participants were retrieved and tested for anti-Bbsl antibodies. Participants ages ranged from 18 −87 years (median age: 45 years); 1037 (51.9 %) of the donors were female (Table 1). Among the tested samples, 1496 (74.9 %) were collected from participants in Bohemia (500 in Ceske Budejovice and 996 in Hradec Kralove) and 500 (25.0 %) in Moravia (Brno). The number (percent) of the tested samples by age group were: 408 (20.4 %) 18 −29 years-of-age, 398 (19.9 %) 30 −29 years-of-age, 370 (18.5 %) 40 −49 years-of-age, 320 (16.0 %) 50 −59 years-of-age, 361 (18.1 %) 60 −69 years-of-age, and 139 (7.0 %) ≥ 70 years-of-age.

In first-tier testing of the 1996 samples for anti-Bbsl antibodies, 112 (5.6 %), 274 (13.7 %), and 359 (18.0 %) were positive for IgM, IgG, and IgM/IgG antibodies, respectively (Supplementary Table 1). In second-tier testing, there were 74, 126, and 190 samples positive for IgM, IgG, and IgM/IgG antibodies, respectively, yielding a prevalence of anti-Bbsl antibodies for IgG of 6.3 % (5.3 −7.5 %), and for IgM/IgG of 9.5 % (8.3 −10.9 %) (Table 2). The seroprevalence was higher in males than in females; IgM/IgG seroprevalence was 12.2 % in males and 7.0 % in females (*p* value <.0001). The seroprevalence increased by age groups with the highest seroprevalence in persons ≥ 70 years-of-age and lowest in persons 18 −29 years-of-age. Among the blood donation centers, the seroprevalence was highest among donors at the Brno center in Moravia and lowest among donors at the Hradec Kralove center in Bohemia (Supplementary Table 1). The IgM/IgG seroprevalence was 7.8 % (6.5 −9.2 %) and 15.3 % (12.2 −19.0 %) among residents of Bohemia and Moravia, respectively (Fig. 1).

In the base case analysis, using the IgM/IgG seroprevalence with a 37 % symptomatic proportion and 10-year duration antibody detection, there were an estimated 30,563 (26,550 −34,962) symptomatic incident Bbsl infections in adults in the Czech Republic in 2012, for an incidence of symptomatic Bbsl infections in adults of 352.2 (306.0 −402.9) per 100,000 PPY (Table 3). By geographic region, Bohemia had a lower incidence of symptomatic Bbsl infections in adults (287.1/100,000 PPY) than Moravia (567.2/100,000 PPY).

There were 2751 surveillance-reported LB cases in adults in 2012, for an incidence of 31.7/100,000 PPY. Of the surveillance-reported LB cases, 1814 were from Bohemia and 937 were from Moravia, for an estimated incidence of surveillance-reported LB cases in adults of 31.9/100,000 PPY in Bohemia and 31.3/100,000 PPY in Moravia. When compared to the estimated incidence symptomatic Bbsl infections in

Table 1
Characteristics of blood donors in seroprevalence study, by blood donation center and region of blood donation center, Czech Republic, 2012.

Characteristics of donors	Ceske Budejovice (Bohemia)	Hradec Kralove (Bohemia)	Brno (Moravia)	All
Sex (n, %)				
Female	271 (54.2)	505 (50.7)	261 (52.2)	1037 (51.9)
Male	229 (45.8)	491 (49.3)	239 (47.8)	959 (48.1)
Age in years				
Range	18–81	18–87	18–85	18–87
Mean (SD)	45.2 (15.9)	45.4 (16.7)	45.3 (16.4)	45.3 (16.4)
Median	45	45	45	45
Age group in years (n, %)				
18–29	102 (20.4)	203 (20.4)	103 (20.6)	408 (20.4)
30–39	94 (18.8)	197 (19.8)	107 (21.4)	398 (19.9)
40–49	106 (21.2)	192 (19.3)	72 (14.4)	370 (18.5)
50–59	73 (14.6)	154 (15.5)	93 (18.6)	320 (16.0)
60–69	100 (20.0)	170 (17.1)	91 (18.2)	361 (18.1)
70+	25 (5.0)	80 (8.0)	34 (6.8)	139 (7.0)
Residence location (n, %)				
Central Bohemia	3 (0.6)	11 (1.1)	4 (0.8)	18 (0.9)
Western/Southern Bohemia	488 (97.6)	0 (0)	6 (1.2)	494 (24.7)
Northern Bohemia	3 (0.6)	2 (0.2)	5 (1.0)	10 (0.5)
Eastern Bohemia/ Western Moravia	1 (0.2)	981 (98.5)	29 (5.8)	1011 (50.7)
Southern Moravia	3 (0.6)	1 (0.1)	406 (81.2)	410 (20.5)
Eastern/Northern Moravia	2 (0.4)	1 (0.1)	50 (10.0)	53 (2.7)
Overall	500	996	500	1996

Table 2
Prevalence of anti-*Borrelia burgdorferi* sensu lato antibodies among blood donors, Czech Republic, 2012.

a. Prevalence of anti-*Borrelia burgdorferi* sensu lato IgG antibodies

Characteristics of donors	Number tested	Number positive	Seroprevalence (95 % CI) of anti-Bbsl IgG antibodies
Sex			
Female	1037	42	4.0 % (2.9 −5.4)
Male	959	84	8.8 % (7.0 −10.7)
Age group in years			
18 −29	408	11	2.7 % (1.3 −4.8)
30 −39	398	16	4.0 % (2.3 −6.4)
40 −49	370	14	3.8 % (2.1 −6.3)
50 −59	320	25	7.8 % (5.1 −11.3)
60 −69	361	40	11.1 % (8.0 −14.8)
70 +	139	20	14.4 % (9.0 −21.3)
Blood donation center			
Ceske Budejovice (Bohemia)	500	25	5.0 % (3.3 −7.3)
Hradec Kralove (Bohemia)	996	51	5.1 % (3.8 −6.7)
Brno (Moravia)	500	50	10.0 % (7.5 −13.0)
Area of residence			
Bohemia	1533	77	5.0 % (4.0 −6.2)
Moravia	463	49	10.6 % (7.9 −13.7)
Overall	1996	126	6.3 % (5.3 −7.5)

b. Prevalence of anti-*Borrelia burgdorferi* sensu lato IgM and/or IgG antibodies

Characteristics of donors	Number tested	Number positive	Seroprevalence (95 % CI) of anti-Bbsl IgM/IgG antibodies
Sex			
Female	1037	73	7.0 % (5.6 −8.8)
Male	959	117	12.2 % (10.2 −14.4)
Age group in years			
18 −29	408	19	4.7 % (2.8 −7.2)
30 −39	398	31	7.8 % (5.3 −10.9)
40 −49	370	28	7.6 % (5.1 −10.7)
50 −59	320	37	11.6 % (8.3 −15.6)
60 −69	361	52	14.4 % (10.9 −18.5)
70 +	139	23	16.5 % (10.8 −23.8)
Blood donation center			
Ceske Budejovice (Bohemia)	500	41	8.2 % (5.9 −11.0)
Hradec Kralove (Bohemia)	996	77	7.7 % (6.1 −9.6)
Brno (Moravia)	500	72	14.4 % (11.4 −17.8)
Area of residence			
Bohemia	1533	119	7.8 % (6.5 −9.2)
Moravia	463	71	15.3 % (12.2 −19.0)
Overall	1996	190	9.5 % (8.3 −10.9)

Bbsl, *Borrelia burgdorferi* sensu lato; CI, confidence interval.

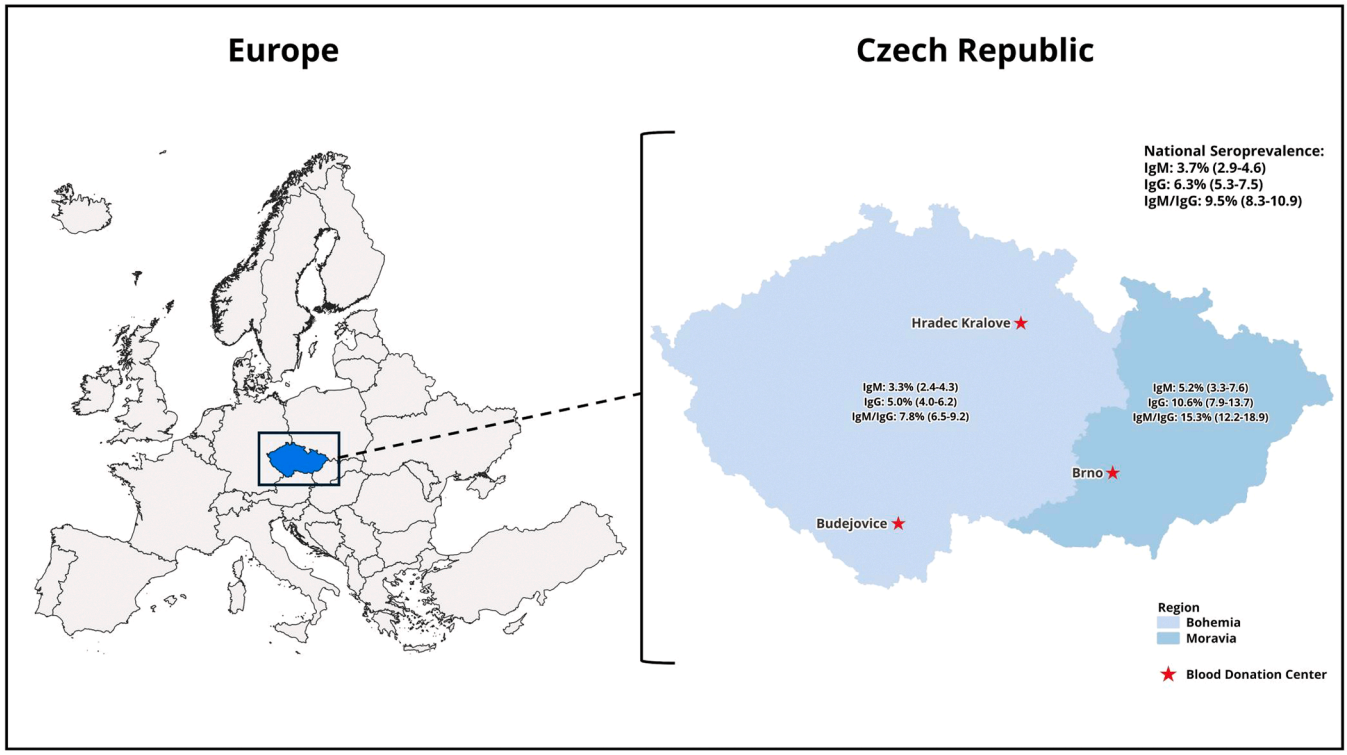

Fig. 1. Location of the blood donation centers and prevalence of anti-*Borrelia burgdorferi* IgM, IgG, and IgM/IgG antibodies in Bohemia and Moravia, Czech Republic, 2012.

Table 3
Estimated incidence (per 100,000 population per year) of symptomatic *Borrelia burgdorferi* infection in adults by area of residence, Czech Republic, 2012.

a. Base case: derived using IgM/IgG seroprevalence

Donor's area of residence	Population ≥ 18 years-of-age	Sero-prevalence of IgM/IgG antibodies (95 % CI)	Symptomatic incident Bbsl infections in adults in 2012* (95 % CI)	Incidence of symptomatic Bbsl infection in adults in 2012 (95 % CI)	Incidence of surveillance-reported Lyme borreliosis cases in adults in 2012	Under-ascertainment multiplier of symptomatic Bbsl infections in adults (95 % CI)
Bohemia	5681,725	7.8 % (6.5 –9.2)	16,313 (13,601 –19,383)	287.1 (239.4 –341.1)	31.9	9 (7 –11)
Moravia	2995,170	15.3 % (12.2 –18.9)	16,989 (13,487 –21,001)	567.2 (450.3 –701.2)	31.3	18 (14 –22)
Nationwide	8676,895	9.5 % (8.3 –10.9)	30,563 (26,550 –34,962)	352.2 (306.0 –402.9)	31.7	11 (10 –13)

b. Sensitivity analysis: derived using IgG seroprevalence

Donor's area of residence	Population ≥ 18 years-of-age	Sero-prevalence of IgG antibodies (95 % CI)	Symptomatic incident Bbsl infections in 2012* (95 % CI)	Incidence of symptomatic Bbsl infection in 2012 (95 % CI)	Incidence of surveillance-reported Lyme borreliosis cases in 2012	Under-ascertainment multiplier of symptomatic Bbsl infections (95 % CI)
Bohemia	5681,725	5.0 % (4.0 –6.2)	10,553 (8367 –13,118)	185.7 (147.3 –230.9)	31.9	6 (5 –7)
Moravia	2995,170	10.6 % (7.9 –13.8)	11,725 (8788 –15,238)	391.5 (293.4 –508.8)	31.3	13 (9 –16)
Nationwide	8676,895	6.3 % (5.3 –7.5)	20,258 (16,983 –23,982)	233.5 (195.7 –276.4)	31.7	7 (6 –9)

* Assuming a 37 % symptomatic proportion and 10-year duration of anti-*Borrelia burgdorferi* sensu lato IgG antibodies. Bbsl, *Borrelia burgdorferi* sensu lato; CI, confidence interval.

adults, there were 11 (10 –13) symptomatic Bbsl infections for each surveillance-reported LB case in adults in 2012 in the Czech Republic; 9 (7 –11) and 18 (14 –22) symptomatic Bbsl infections for each surveillance-reported LB case in adults in Bohemia and Moravia, respectively. In the sensitivity analysis, using the IgG seroprevalence, there were an estimated 20,258 (16,983 –23,982) symptomatic incident Bbsl infections in adults in the Czech Republic in 2012, for an incidence of symptomatic Bbsl infections in adults of 233.5 (195.7 –276.4) per 100,000 population per year. When compared to the incidence of surveillance-reported LB cases in adults in the sensitivity analysis, there were 7 (6 –9) symptomatic Bbsl infections in adults for each surveillance-reported LB case in adults in 2012 in the Czech Republic; 13 (9 –16) in Moravia compared to 6 (5 –7) in Bohemia.

4. Discussion

In the largest nationwide general population LB seroprevalence study conducted in the Czech Republic to date, we found a prevalence of anti-Bbsl IgG/IgM antibodies of 9.5 % in samples collected in 2011 –2012 from almost two thousand adults. The seroprevalence was higher in Moravia (15.3 %) than Bohemia (7.8 %). Using the seroprevalence data, the estimated incidence of symptomatic Bbsl infection in the Czech Republic was 352 per 100,000 adults in 2012, a substantial burden of LB in the Czech Republic, particularly in Moravia.

The seroprevalence of anti-Bbsl IgG antibodies in our study in the Czech Republic (6.3 %) is comparable to other general population seroprevalence studies conducted in LB endemic countries such as Germany (9.4 %) and Finland (3.9 %) (Wilking et al., 2015; van Beek

et al., 2018). The high anti-Bbsl seroprevalence in the Czech Republic is associated with a high incidence of surveillance-reported LB cases (Orlíková et al., 2022), and high incidence of LNB cases (Kříž et al., 2017). A previous seroprevalence study in the Czech Republic, which only tested for anti-Bbsl IgG antibodies at the NRL, used sera collected from 434 and 270 members of the general population in 1979 and 2001, respectively, and reported a prevalence of anti-Bbsl IgG antibodies of 25.1 % in 1979 and 10.4 % in 2001 (Kříž et al., 2018a). The lower seroprevalence observed in our study may be related to the availability and use of more specific ELISA tests or to increased diagnosis and treatment of Lyme disease cases in the Czech Republic (Kříž et al., 2018b).

Our study is the first to report the prevalence of anti-Bbsl IgM/IgG antibodies in the Czech Republic. The prevalence of anti-Bbsl IgM/IgG antibodies (9.5 %) is higher than the prevalence of anti-Bbsl IgG antibodies alone (6.3 %) because some participants had only anti-Bbsl IgM antibodies. The presence of IgM antibodies usually suggests recent Bbsl infection as IgM antibodies are produced early in infection (Aguero-Rosenfeld et al., 2005). However, anti-Bbsl IgM antibodies can persist in individuals for longer than six months after infection, including when IgG are present (Markowicz et al., 2021). The high prevalence of anti-Bbsl IgM/IgG antibodies identified in our study suggest frequent exposure to Bbsl-infected ticks in the Czech Republic, particularly in Moravia, which includes the four most eastern regions in the country. Our results also show that individuals with anti-Bbsl IgM antibodies represent a substantial proportion of those with anti-Bbsl antibodies. Of note, several general population Lyme seroprevalence studies in other countries did not test for IgM antibodies, potentially leading them to underestimate seroprevalence (Wilking et al., 2015; van Beek et al., 2018).

Public health surveillance for LB was established in the Czech Republic in the mid-1980s and has shown an increase in the incidence of surveillance-reported cases in recent years (Orlíková et al., 2022). Climate change has been postulated to be a key contributor to the increased incidence (Daniel et al., 2009; Danielová et al., 2010; Zeman and Benes, 2013). Evaluations of the Czech Republic surveillance system for LB suggest that it is comprehensive (Orlíková et al., 2022). For example, there was a notably higher incidence of surveillance-reported LB cases in the regions of the Czech Republic than in the bordering regions of Poland, indicating more complete reporting of LB cases (Stefanoff et al., 2014). Despite the comprehensive nature of the Czech Republic surveillance system, results from our study estimate that there are eleven symptomatic Bbsl infections for each surveillance-reported LB case in the Czech Republic. Furthermore, under-ascertainment of symptomatic Bbsl infections by public health surveillance was greater in the eastern area of Moravia, where there were eighteen symptomatic Bbsl infections for each surveillance reported LB case. The estimated under-ascertainment of LB cases by surveillance in the Czech Republic is further supported by the proportion of surveillance-reported LB cases with different clinical manifestations; in the Czech Republic, 62 −67 % of surveillance-reported cases are EM cases and 12 −25 % are LNB cases (Orlíková et al., 2022; Kříž et al., 2018b). In contrast, in several other European countries, > 90 % of surveillance-reported cases are EM and only 2 −4 % LNB (Brestrich et al., 2024; Geebelen et al., 2019; Hofhuis et al., 2015; Paradowska-Stankiewicz et al., 2023; Septfons et al., 2019). Although differences in surveillance approaches make it difficult to compare LB surveillance data between countries, the low proportion of surveillance-reported cases that are EM in the Czech Republic suggests that surveillance may miss a substantial number of EM cases.

This study is subject to several limitations. The blood samples tested in our study were collected from participants in 2011 −2012 but were not tested for anti-Bbsl antibodies until 2024. We assumed that there would not be a decrease in anti-Bbsl antibodies in the specimens during the 12 years of storage since the specimens were maintained at −20°C. To confirm this assumption, we tested a subset of the specimens which were previously positive for anti-Bp antibodies and found no decline in anti-Bp antibodies. Therefore, we believe sample quality was preserved and that there was no general decline in antibodies including anti-Bbsl antibodies during storage. Another limitation is that we generalized the results from the testing of aliquot sera collected from donors at three donation centers to the entire Czech Republic. The centers were geographically dispersed throughout the country supporting extrapolation of study results nationwide, but the data were not robust enough to support seroprevalence estimates at the regional level other than for Bohemia and Moravia. A further limitation of our study is that the estimate of the incidence of symptomatic Bbsl infections relies on an estimate of the proportion of Bbsl-infected persons who have symptoms and an estimate of the duration of detection of ant-Bbsl IgG antibodies (Angulo et al., 2024). Both estimates, particularly the duration of IgG antibodies in Bbsl-infected individuals, are subject to uncertainty (Angulo et al., 2024). We used a 37 % estimate of the symptomatic proportion, if a lower estimate of the symptomatic proportion was used, the estimated incidence of symptomatic Bbsl infections would be reduced. Similarly, we used a 10-year duration of persistence in our estimation; if a longer persistence was used, the estimated incidence of symptomatic Bbsl infections would be reduced. Further studies to estimate the symptomatic proportion and duration of persistence of IgG antibodies in Bbsl-infected persons would be helpful for estimating the incidence of symptomatic Bbsl infections from seroprevalence studies.

5. Conclusions

There is a substantial seroprevalence of anti-Bbsl antibodies in the Czech Republic, indicating a high incidence of symptomatic Bbsl infections. Comparing symptomatic Bbsl infections to surveillance-reported LB cases and clinical manifestations indicates that public health surveillance significantly under-ascertains EM cases. Interventions are needed to address the high burden of LB in the Czech Republic.

Ethical approval

The protocol for this study was approved by the multicentric Ethics Committee of the University Hospital of Hradec Kralove (Sokolská 581, 500 05 Nový Hradec Kralove) on September 21, 2023 (reference number 202309P04).

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Declaration of Competing Interest

Frederick J. Angulo, Alexandra Loew-Baselli, Ye Tan, Aleš Ondřejíček, Gordon Brestrich, Andreas Pilz, Jennifer C. Moïsi, and James H. Stark are employees of Pfizer and may hold stock or stock options.

Author statement

Roman Chlibek and Frederick J. Angulo wrote the manuscript. Roman Chlibek, Jan Smetana, Kateřina Kybicová, Martina Malikova, Frederick J. Angulo, and Alexandra Loew-Baselli planned and coordinated the study. Roman Chlibek, Jan Smetana, Kateřina Kybicová, and Martina Malikova were responsible for data collection. Roman Chlibek, Jan Smetana, Kateřina Kybicová, Frederick J. Angulo, Alexandra Loew-Baselli, Ye Tan, and Gordon Brestrich were responsible for data analysis, figure production, and writing of the methods. Roman Chlibek, Jan Smetana, Kateřina Kybicová, Frederick J. Angulo, Alexandra Loew-Baselli, and Ye Tan had direct access and verified the data reported in the manuscript. All authors reviewed the method and preliminary

findings. All authors critically revised the manuscript.

CRediT authorship contribution statement

Alexandra Loew-Baselli: Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Resources, Project administration, Methodology, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Ye Tan:** Writing – review & editing, Visualization, Validation, Methodology, Investigation, Formal analysis, Data curation. **Aleš Ondřejíček:** Writing – review & editing, Supervision, Resources, Conceptualization. **Gordon Brestrich:** Writing – review & editing, Validation, Supervision, Methodology, Investigation, Formal analysis, Conceptualization. **Andreas Pilz:** Writing – review & editing, Supervision, Resources, Methodology, Funding acquisition, Conceptualization. **Jennifer C. Moïsi:** Writing – review & editing, Supervision, Resources, Funding acquisition. **James H. Stark:** Writing – review & editing, Resources, Funding acquisition, Conceptualization. **Frederick James Angulo:** Writing – review & editing, Writing – original draft, Visualization, Supervision, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Conceptualization. **Roman Chlibek:** Writing – review & editing, Writing – original draft, Supervision, Project administration, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Jan Smetana:** Writing – review & editing, Writing – original draft, Validation, Supervision, Project administration, Methodology, Investigation, Data curation. **Kateřina Kybicová:** Writing – review & editing, Validation, Supervision, Project administration, Methodology, Investigation, Formal analysis, Conceptualization. **Martina Malikova:** Writing – review & editing, Supervision, Software, Project administration, Methodology, Investigation, Data curation.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.ijmm.2024.151644.

Data availability

Data will be made available on request.

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